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PROGRESS REPORT
of the
NORTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION
AGRICULTURAL RESEARCH SERVICE

This progress report includes a summary of the current research of the Division and a preliminary report of progress made during the preceding year. It is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1967, and June 30, 1968. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois.

UNITED STATES DEPARTMENT OF AGRICULTURE

Washington, D. C.

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INTRODUCTION

The Northern Utilization Research and Development Division, located at Peoria, Illinois, is one of five research divisions of the Agricultural Research Service concerned with the development of basic knowledge of chemical composition and physical properties of farm commodities and with the application of this knowledge to the development of new or improved products and processing technology that will enhance utilization of these commodities. The other Utilization Research and Development Divisions are the Eastern at Philadelphia, Pennsylvania, the Southern at New Orleans, Louisiana, the Southeastern at Athens, Georgia, and the Western at Albany, California.

The need and importance of utilization research on farm commodities arise from the fact that the farmer is not organized to carry on modern scientific research to maintain old, and create new, markets for his products. The Northern Division is responsible for the Department's utilization research on corn, grain sorghum, soybeans, flax, crambe, and new crops. Its research on wheat emphasizes industrial utilization and milling technology, and that on forages is limited to a search for the cause of toxicity occasionally displayed by tall fescue grass. Responsibility for research on food and feed utilization of wheat and for the Department's primary utilization research program on forages is assigned to the Western Division.

The scientific research effort at the Northern Division amounts to approximately 192 scientist man-years. In addition, the Division supervises domestic research contracts equivalent to 13.8 scientist man-years and grants equivalent to 20.3 scientist man-years, and sponsors a comprehensive program of research comprising 28 PL 480 grants to foreign institutions.

Following are some of the recent utilization research accomplishments of the Northern Division.

Starch-based reinforcing agents for rubber--a new technology. In studies to increase industrial uses for cereal starches, Department scientists have developed three starch-based reinforcing agents for rubber (zinc starch xanthate, starch xanthide, and starch-resorcinol-formaldehyde resin). Laboratory-scale evaluations show that these starch products are comparable to medium-grade carbon black and superior to common clays in improving tensile strength and most other physical properties of natural and synthetic rubbers. All three of the starch products are incorporated into rubber by minor variations of a simple basic process which is compatible with current practices in the industry. White or brightly colored rubbers which exceed the strength of white rubber now used in white sidewall tires and shoes can be readily made with the starch xanthate and xanthide. The starch-resin product gives tough, strong, dark-colored rubber similar to that obtained with carbon black.

On the basis of properties and estimated costs, the starch-reinforced rubbers should be competitive with conventional rubbers in a number of applications and superior in others. At present 100-pound batches of selected starch-reinforced rubbers are being made up for thorough evaluation as general-purpose molding compounds, white sidewalls for tires, tire carcass compounds, shoe soling, and fuel-resistant hose. Current annual domestic consumption of black and nonblack reinforcing agents in rubber is approximately 5 billion pounds; thus, the potential market for starch-based reinforcing agents is exceedingly large. This new technology has created more domestic and worldwide interest than any other development by the Northern Division over the past several years. Two patent applications have been filed to cover the new technology involved.

Wheat starch achieves major market in Pacific Northwest. A process developed by Department scientists that separates wheat flour into starch and food-grade vital gluten served as a basis for a recently completed plant in the Pacific Northwest. For the first time, industrial wheat starch is available in that area and is being used by the regional pulp and paper industry. At least three large paper companies now use multi-million pounds of wheat starch annually.

The company producing the starch consulted extensively with Department scientists on the plant process design; on the physical and chemical properties of wheat flour and its mechanical behavior; and on the processing and application techniques for wheat starch in paper.

Five other United States wheat starch plants located elsewhere are largely based on the Department-developed process. Previously, wheat starch has primarily been used in food and specialty applications.

Processing corn fractions for use in food blends. Department engineers have developed processing conditions for precooking corn endosperm fractions by means of an extrusion cooking system to yield a product which is useful in gruel mixes. Processing or precooking of corn grits, meal, or flour is necessary when the corn product is to be used in food blends that are partially or completely precooked. As a direct result of this work, at least three commercial corn processors have been able to use their extrusion equipment for producing partially precooked corn of the proper characteristics for use in CSM (corn, soya, milk food blend) for distribution abroad by the voluntary agencies. The corn fraction constitutes over 60 percent of the total blend. CSM is an excellent protein supplement in the diets of weanling and preschool-age children. Use of these processed corn products in the donation programs alone could easily amount to as much as 300 million pounds per year, corresponding to about 10 million bushels of corn annually.

Survey for aflatoxin in grains. A survey by Department scientists showed that very little or no aflatoxin occurs in grains in commercial trade channels. In this survey 3,545 samples of corn, oats, wheat, soybeans, and grain sorghum representing all USDA Grain Inspection Service grades were tested for the presence of aflatoxins. Samples were collected at selected terminal markets from October 1964 to November 1966 except corn which was also collected from the 1967 crop. To assure maximum significance of results, the number of samples of each kind and grade of grain was based on the proportions of each grade as reported. The presence of aflatoxins, carcinogenic toxins produced by mold, was not confirmed by biological tests in any of the wheat, grain sorghum, or oat samples. Only 2 out of 866 soybeans contained aflatoxin and they were at very low levels. Both chemical and biological tests showed that none of the corn samples in Grades 1, 2, 3, and 4 contained aflatoxin; but 5 out of 281 samples in Grade 5 and 25 out of 353 Sample Grade corn had low levels of aflatoxin. Factors that place corn in Sample Grade are moldiness, mustiness, off color, and off odor. This survey showed that it is important to keep the poorest grades separate from better grades and not to use them for food or feed purposes.

Aflatoxin standards for the feed and food industries. Crystalline aflatoxin standards prepared by Department scientists were made available at no cost to research and control laboratories in the feed and food industry. Aflatoxin is a toxic cancer-inducing material produced by certain molds growing on agricultural commodities. Very small amounts of aflatoxin are carcinogenic. The crystalline samples of the toxin are required as standards for the detection and identification of the aflatoxins occurring in food and feed. Molds were grown in the laboratories, and the toxin was isolated and purified. One hundred thirty-six samples were supplied to industrial laboratories, research institutes, and government laboratories. The Department is now the only source of this material in the world and, consequently, has supplied standards to 25 different countries. In addition, 5 grams of the most toxic aflatoxin and 5 grams of mixed toxins were supplied to other research organizations for studies on animal nutrition, tumor formation, and means of detoxification. An earlier supplier of aflatoxin reference standards, who no longer offers it, quoted it at \$25 per milligram. On this basis, the material the Department has supplied has a value well over \$100,000.

Rapid extrusion cooking process for full-fat soy flour. A new process, based on mechanical extrusion cooking, was developed by Department engineers for the manufacture of a high-quality full-fat soy flour for food use. This work was initiated in cooperation with UNICEF and later completed under a PASA agreement with AID. In this process, the dehulled flakes or grits are preconditioned by dry heat before going into the extruder-cooker where they are exposed to higher temperatures for a short time. Almost instantaneous cooking is achieved with minimum damage to heat-sensitive nutritional factors.

UNICEF has reported that deficiencies in the diet of both children and adults in many developing countries is not just a lack of proteins but also a shortage of calories. Therefore, full-fat (unextracted) soy flour containing high-caloric-value oil, in addition to a high concentration of high-quality protein, is desirable for formulation with indigenous foods into products with high nutritional value. Extrusion-cooking plants have been installed in Taiwan, Hong Kong, Venezuela, and South Africa. Equipment suitable for extrusion cooking is being produced and promoted commercially by a Midwest company. United States food processors are showing considerable interest in the process. Also, approximately 14 plants in England are producing extruded full-fat soybean meals for pet foods.

Commercial process for crambe meal for cattle feed. Department scientists have shown how to make a protein feed supplement suitable for cattle feeding from crambe seed, a new crop development. High-quality crambe oil is obtained at the same time. The process is based on soda ash treatment of the crambe seed, which modifies undesirable thioglucosides and improves palatability. Cooperative feeding tests at the University of Nebraska Agricultural Experiment Station have demonstrated the improved palatability and nutritional quality of this meal for the finishing of beef cattle. When used with soybean meal to provide up to 50 percent of the protein supplement, it gave results equal to those with all soybean meal supplement. Further research is needed to make the meal suitable for feeding nonruminants. Crambe seed was first grown as a new commercial crop in the United States in 1965 and the first crambe meal made commercially available in the United States was by a modified soda ash treatment that fall. This meal was successfully marketed for use in cattle feed. Foreseeable demand for crambe oil is estimated at 200 million pounds per year. This production will require 300,000 tons of crambe seed annually with a farm value of nearly \$25 million annually on a farm acreage of 400,000 acres.

A new nylon from an agricultural raw material. A new nylon with outstandingly useful properties has been prepared under the Department's research program on new crops. Laboratory studies show that this new product, called nylon 1313, is easily made from crambe seed oil. Crambe is a new oilseed being developed as a new crop for the United States. Nylon 1313 is very tough, has low moisture absorption, and flows easily at lower temperatures than textile-type nylons. These characteristics are valuable for metal coatings and adhesives, bearings, electrical insulators, brush bristles, food-wrapping films, and other molded and extruded articles. Nylon 1313 samples have been requested by more than 36 companies for evaluation in specific applications. Demonstrated ease of laboratory preparation and the favorable properties found for nylon 1313 provide a needed foundation for development work directed toward commercialization.

Kenaf for paper pulp. Department research has shown that both green kenaf, as early as 120 days after planting, and dry kenaf produce high-quality paper pulp. Therefore, kenaf can be harvested and used during many months without expensive storage or preprocessing. The suitability of green kenaf for pulp is especially advantageous in the South where it stays green until late fall.

Kenaf is a high-yielding new annual crop that grows well in the Southern regions of the United States. Test plantings have given fiber production two or three times greater than fast-growing woods. The pulp characteristics are equal to or superior to hardwood pulp.

The major paper companies are now evaluating the production and utilization of kenaf based on Department findings. Over 30 industrial companies participated in a technical conference on it in 1967. A special committee of industry and government scientists has been set up by the Technical Association of Pulp and Paper Industries to assist in the overall evaluation.

Valuable breadmaking ingredient from quaking grass seed. Department scientists have found that seeds of a Turkish species of quaking grass, called Briza spicata, contain a substance that increases the volume of a loaf of bread. Loaf-volume expanders are prized by breadmakers since they permit favorable crumb structures with a wide range of flours and with breads enriched in protein content. Consumer appeal is also enhanced. A similar material with both sugars and fatty portions combined in a single complex molecule occurs in very small amount in wheat. However, quaking grass seed was found in USDA's new crops program to be the richest known source of the substance. As a bread ingredient, only 0.2 percent of the seed substance from Briza increases loaf volume as much as one-fifth. Inquiries have been received from companies that wished to obtain substantial amounts of Briza seed for evaluation or to make their own plantings. Preliminary agronomic evaluation of quaking grass is favorable. Cultivation of quaking grass as a new United States crop to furnish this naturally occurring additive for use in commercial breadmaking should provide an attractive benefit for both farmers and processors.

CORN UTILIZATION

USDA and Cooperative Programs

Location and Nature of Intramural Work	Scientist Man-Years FY 1968:				Total
	Research Problem Area				
	406	407	702	901	
Peoria, Illinois					
<u>Food</u>					
A. Chemical Composition and Physical Properties	9.7				9.7
B. Color, Texture and Other Quality Factors	2.5				2.5
C. Microbiology and Toxicology	0.9		6.5		7.4
D. Technology--Process and Product Development	1.8				1.8
<u>Industrial Products</u>					
A. Chemical Composition, Physi- cal Properties and Structure		9.0			9.0
B. Chemical and Physical Investi- gations to Improve Products		12.0		1.8	13.8
C. Microbiology and Fermentation		9.2			9.2
D. Technology--Process and Product Development		5.6			5.6
Total	14.9	35.8	6.5	1.8	59.0

Intramural program is supplemented by extramural support representing (a) 0.7 SMY's at State Agricultural Experiment Stations^{1/}, (b) 13.4 SMY's at other U. S. institutions^{2/}, and (c) P.L. 480 funds in 10 countries representing 975,423 U. S. dollars equivalent.

- ^{1/} RPA 407: 0.3 (Chemical and Physical Investigations to Improve Products)
RPA 702: 0.4 (Microbiology and Toxicology)
- ^{2/} RPA 406: 3.6 (Chemical Composition and Physical Properties, 1.3; Microbiology and Toxicology, 2.3)
RPA 407: 8.7 (Chemical Composition, Physical Properties and Structure, 1.2; Chemical and Physical Investigations to Improve Products, 2.7; Microbiology and Fermentation, 1.5; Technology--Process and Product Development, 3.3)
RPA 702: 0.2 (Microbiology and Toxicology)
RPA 901: 0.9 (Chemical and Physical Investigations to Improve Products)

Problems and Objectives

The farmer sells 100,000,000 bu. of grain to make 3 billion pounds of cereal starches and flours that are used annually in the U.S. for industrial purposes. Corn is the source of most of these products. These outlets are constantly threatened by synthetic products derived from non-agricultural sources. Increasing the present and future competitive position of corn starch and flour in industrial markets requires development of new and improved products that will do a better job in outlets such as the paper industry, industrial chemicals, adhesives, protective coatings, plastics, elastomers, and thickening agents. Almost 80 percent of the U.S. annual production of corn is used as animal feed. Because of the extremely large volume of this outlet, even small improvements in quality or processing efficiency give direct gains to industry and to the farmer. Utilization of corn in foods also is an outlet of great economic importance. Significant benefits can be realized if we can make cheaper and more nutritious products, including foods from new corn varieties having higher nutritional value than does ordinary dent corn.

Major objectives of the current research are to develop and evaluate alternate ways to:

1. Discover new technology for converting starch by chemical or fermentative means to products for use in the paper, chemical, and other industries.
2. Provide increased industrial outlets for new classes of high-amylose corn.
3. Improve yields and quality of corn dry milling fractions.
4. Lower the cost of converting corn to sugars and sirups and to more acceptable, varied, and nutritious foods.
5. Remove mold toxins or harmful microorganisms from corn and corn products.

Progress - USDA and Cooperative Programs

RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

A. Chemical Composition and Physical Properties

1. Corn proteins. Gel filtration chromatography on Sephadex G-100 at a pH of 8.6 has yielded several fractions from germ and endosperm albumins. Extensive aggregation at this pH was evidenced by the elution volume of the bulk of the proteins in contrast to results at pH 4.4. Subcellular particles from immature corn endosperm homogenates in phosphate buffer were

separated by ultracentrifugation on a sucrose density gradient. The major protein of the protein bodies was identified as zein. Removal of the zein with 70 percent ethanol extraction resulted in a residual protein with unique amino acid and electrophoretic properties after reduction. This protein resembles certain of the glutelin components isolated by direct extraction from endosperm. Amino acid and protein analyses of high-lysine corn kernel fractions showed that germ cake, bran, and aspiration meal have high protein content and good amino acid balance. The aleurone layer, comprising 2 percent of the grain, contained 30 percent protein, predominantly albumins and globulins rich in lysine.

Various methods of tryptophan analysis have been evaluated. S(pyridyl-ethyl)-L-cysteine has been prepared and characterized for use as an internal standard in amino acid analysis. A computer program, using linear programming, has been written which gives the best nutritionally optimum combination of cereal grains based upon their essential amino acid patterns and cost.

Grant research at Purdue University, Lafayette, Indiana, showed that water-soluble proteins of corn are separable by chromatography on DEAE-cellulose columns. An improved method for tryptophan analysis that provides accurate reproducible values has been developed which will allow routine analysis for this amino acid in corn. A larger amount of dialyzable nitrogen extracted with saline solution from high-lysine corn than from normal corn was found to be due to a high level of nonprotein nitrogen. Most of this nonprotein nitrogen was attributable to free amino acids.

2. Corn lipids. Two hybrid yellow corns were hand-dissected into pericarp (4% of the whole kernel), tip cap (4%), floury endosperm (22%), horny endosperm (63%), and germ (7%). Hexane-soluble material in the whole corn averaged 3.6 percent. Thin-layer chromatograms of nonpolar lipids and the hand-dissected fractions were scanned by a densitometer. Average values for the various lipid fractions as percent of the total lipids in the hexane extract were: phospholipids, 1; diglycerides, 3; sterols, 3; unknown "A", 2; fatty acids, 6; unknown "B", 5; triglycerides, 73; and hydrocarbons and sterol esters, 7. In the hand-dissected fractions, unknown "A", diglycerides and sterols made up 1 to 6 percent of the lipids. Fatty acids ranged from 2 to 14 percent and unknown "B" from 0.5 to 12 percent. Triglycerides, the major fraction, accounted for 55 to 88 percent of the total lipids. Hydrocarbons and sterol esters were five times more concentrated in the horny endosperm than in the floury endosperm.

3. Reactions of maltose and glucose. Intermolecular hydrogen-bonded hydroxyl groups, as shown by X-ray structural studies of maltose, lactose, and cellobiose, persist in lowly polar solvents and now appear to be detectable by chemical methods. Heterogeneous acetylation of α - or β -maltose gave a 70-percent yield of 1,2,6,2',3',4',6'-hepta-O-acetyl maltose with only a 25-percent yield of the octaacetate. The corresponding heptaacetates of α - and β -lactose and of β -cellobiose were formed in 50-percent yield. Iso-maltose and melibiose (not intramolecularly hydrogen-bonded) were almost completely acetylated under identical conditions. The α -anomer of

isomaltose, previously unknown, complexes in crystalline form with either sodium iodide or sodium bromide. It was recovered in 92-percent anomeric purity. Large β -maltose hydrate crystals suitable for X-ray structural studies were obtained in 99-percent anomeric purity. Phase diagrams of the melting point of intimate mixtures of anhydrous maltose or D-glucose with urea show that maltose forms 4:1, 2:1, and 1:1 molecular complexes, whereas D-glucose forms only a 1:1 complex. A 1:1 complex of maltose with imidazole was precipitated from ethyl acetate solution, but D-glucose did not give an isolable complex under similar conditions.

4. Structural and chemical differences among corn genotypes. Alcohol solubility data and microscopic analysis have shown that opaque-2 and floury-2 high-lysine maize mutants were deficient in granular protein, the main site of zein deposition in maize endosperm. Subcellular bodies similar to zein bodies of normal maize are lacking in the protein structure of floury-2 mutant. In opaque-2, small protein bodies averaging about 0.1 μ in diameter were noted 14 days after pollination. The size of these bodies was unchanged in samples collected at 20 and 50 days. In contrast, zein bodies of normal corn showed a 10- to 20-fold increase in size over this period. The two mutants responded differently to gene dosage. Endosperm subcellular structure in the floury-2 mutant developed subcellular protein structure similar to the homozygous condition with one dose of the floury-2 gene. In opaque-2, well-developed zein granules persisted in endosperm protein with one and two doses of opaque-2 gene.

5. Phytin in corn. Based on studies of wet- and dry-milled corn products obtained from industrial organizations, preliminary results from grant research at the University of Missouri, Columbia, Missouri, indicate that over one-half of the phytate in corn is present in the germ.

B. Color, Texture and Other Quality Factors

1. Browning reactions. Dry heating of the Amadori compound, 1-deoxy-1-piperidino-D-fructose (a model for cereal browning intermediates), under vacuum at 110° C. gave a sublimate that contained five major and at least eight minor components. Nine compounds were cleanly separated by column and gas chromatography, and their structures were determined by nuclear magnetic resonance, infrared and mass spectrometry, and synthesis. Major components were piperidine acetate, piperidides of carbonic, formic, and acetic acids, piperidino-C-methyl-triose-reductone (enolic 1-piperidino-butanedione-2,3), and α -piperidino- γ -butyrolactone. Minor components were piperidides of glycolic and lactic acids, and an isomeric piperidino-C-methyl-triose reductone. A trace fraction with fragrant caramel aroma was separated into nine components, several of which have been tentatively identified. The amino hexose chain-fission gave predominantly 2- and 4-carbon fragments, and not the 3-carbon fragments that predominate from unsubstituted hexoses in aqueous alkaline solutions. Origin of the most powerfully fragrant caramel aroma compound, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, now detected in several foods, was traced to a 1-amino-1-deoxy-2-ketose (Amadori compound) intermediate.

C. Microbiology and Toxicology

1. Studies on enzymes. In studies on enzymes under a grant to the University of Nebraska, Lincoln, Nebraska, two Aspergillus niger glucoamylases (I, II) showed molecular weights of 125,000 by sedimentation velocity techniques while sedimentation equilibrium analyses indicated 80,600 for I and 58,500 for II. Significantly, this value for II was only about one-half the value of 98,000 obtained by N-terminal amino acid analyses. Carbohydrate moieties of both isozymes appear to be linked through D-mannose to serine or threonine and to be present as small oligomeric units.

Under a grant to the University of Arkansas, Fayetteville, Arkansas, studies on labeled oligosaccharides have permitted the assignment of relative binding affinities to the various subsites on Bacillus subtilis amylase. The active site spans about nine glucose residues and appears relatively rigid. The rate of cleavage of a given glycosidic linkage is presumably independent of the size of the substrate. Attempts to label the active site of lysozyme with radioactive cellobiose appear to be successful.

At Iowa State University, Ames, Iowa, also under grant, a strain of Streptococcus equinus was found which produces an extracellular amylase but is not repressed by glucose as is extracellular amylase production in most Streptococci. Strains of Pseudomonas stutzeri produced maltotetraose almost exclusively from amylose and amylopectin. Ps. saccharophila produces maltose and maltotriose as well as lesser amounts of higher dextrans. Numerous strains of Bacillus have been screened for amylolytic properties. Some appear to resemble pancreatic α -amylase while others yield typical B. subtilis-type α -amylase. Some strains of B. polymyxa produce predominantly maltose as the end products. One polymyxa strain gives oligosaccharides of three and five glucose units in length.

In research on the purification of enzymes, under a grant to Kansas State University, Manhattan, Kansas, Sephadex G-200 Superfine was found to be a satisfactory anti-convection agent for preparative disc electrophoresis. Chromatographic separations of mixed proteins in near gram quantities are now possible. A new column design permits stacking phase separations in which the resolved sample may be removed by liquid flow into fraction collector tubes. A system of electrolyte materials has been developed which employs an intrinsically slow species of unit charge and extends the separating abilities of the system to a wider variety of proteins. The system operates with lower current requirements, thus preventing heating of the columns.

2. Taxonomic and related investigations. Zygosporangia were discovered for the first time for Rhizopus oryzae. NRRL 3133, which is probably Went's type strain for the species, formed up to 277 zygosporangia when mated with three other strains of R. oryzae in the collection. As many as 550 zygosporangia were formed when NRRL 3133 was mated with a strongly mating strain

of R. microsporus, which in turn gave over 1,000 zygospores per plating with NRRL 2915, the type strain of R. microsporus.

Under a grant to the University of Minnesota, Minneapolis, Minnesota, initial work has been primarily concerned with the development of methods. Photographic procedures for studying hyphal growth and methods for determining enzymatic activity of hyphal sections are being established.

D. Technology--Process and Product Development

1. Corn dry milling. Cold tempering and degermination tests on a second lot of high-moisture corn (19%) confirmed previous results indicating a minimum change in degerminator response with change in temper time. Few stress cracks were found in air-dried -4+5 grits prepared from 21-percent-moisture corn under a variety of tempering conditions, a small number from 17-percent corn, and an appreciable number from 13-percent corn. An exploratory, two-step, simulated, series-type operation of tempered corn through a Beall degerminator increased yield of -4+6 grits by 25 percent over a conventional one-pass operation, but did not decrease production of undesirable fines. Treatment of whole untempered corn in a 5-percent caustic solution for 5 minutes followed by passage through a modified sanitary pump successfully removed much of the hull. After tempering and degerminating, the dehulled corn produced 5 percent less fines, 10 percent more grits, and 0.1 to 0.4 percentage point less oil in the grits.

2. Mechanical and viscoelastic properties of corn kernels. In studies at Pennsylvania State University, University Park, Pennsylvania, a slab of horny endosperm (12% moisture, w.b.) from a corn kernel was found to have a complex frequency-dependent modulus (E) of 12,800 pounds per square inch. E, evaluated at 1 cycle/second, was found to decrease with increasing moisture content over the range of 7 to 25 percent (d.b.) with a large drop occurring at the 16-17 percent moisture content level. The expansion coefficient for horny endosperm has been experimentally determined to be approximately three times as great for a 1 percent change in moisture content as for a 1° F. change in temperature. A complex equation has been developed for predicting conditions leading to the formation of undesirable stress cracks during the process for artificially drying corn.

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Industrial Products

A. Chemical Composition, Physical Properties and Structure

1. Physical and chemical studies on starch components. Detailed studies on the optical rotation of starch components in the solvent system H₂O-DMSO, needed for the determination of concentrations in solution, are nearing completion. Experimental studies show a discontinuity in the specific rotation corrected for refractive index variation at a solvent

composition corresponding to the proposed complex $2H_2O$ -DMSO. This discontinuity is exhibited by α -1,4-linked polysaccharides such as corn and potato amylose and amylopectin. Optical rotation studies are underway on model sugars, and hydrodynamic studies on amylose are being made to determine whether the discontinuity corresponds to a polymer conformation change or a solvent effect.

The oriented structure " V_{DMSO} " obtained from dimethyl-sulfoxide-cast amylose films may be transformed to other "V" structures by appropriate chemical treatment. Unit cells and the probable space group of $P2_12_12_1$ were determined for the "V" anhydrous and "V" hydrate forms of amylose. The analysis confirmed previous predictions by Rundle and coworkers. Complexes of amylose with n-butanol were prepared and subjected to X-ray analysis. Their unit cells were calculated and the space group $P2_12_12_1$ was confirmed. No evidence exists for methanol, ethanol, or n-propanol structures similar to that shown by the n-butanol complex.

An NMR method for determining the position of S-benzyl xanthate in a series of α -D-glucopyranoside derivatives has been developed and successfully used to differentiate the 2-, 3-, and 6-substituted isomers of a prepared series. The technique should be applicable to the study of other electronegative substituents. Deuterium resonance has been applied successfully to the problem of determining the deuteration site in experiments carried out under the same conditions as hydrogenation.

Scattering patterns from single starch granules are being investigated under a grant to the State University of New York, Syracuse, New York. Higher order maxima predicted by theory have been observed for canna starch granules dispersed in an imbibition liquid of matching refractive index. Work has begun on preparation of amylose films with both oriented and unoriented specific crystalline structure. An oriented "B" X-ray pattern amylose film is being used to determine the sign of birefringence for the oriented "B" structure.

In studies under a grant to Arizona State University, Tempe, Arizona, it was found that the reaction between "V" amylose hydrate and HCl yielded more constant ratios of products than did the reaction between amorphous amylose and HCl. This result indicates that the sites offered in the crystalline form are more selective in reactions with small molecules. A Patterson function of KOH-amylose is being constructed. As a main feature it has a large peak at $(1/2, 0, 1/2)$ which is interpreted to mean that only a limited number of helix orientations are possible in the $P2_12_12_1$ space group.

Research conducted under a PL 480 grant to the University of Osaka Prefecture, Sakai, Japan, showed that the reaction of o-phenylenediamine with glucose, maltose, and related oligosaccharides gave quinoxalines that can be determined polarographically. Reaction conditions are being sought for applying this reaction to the determination of carbonyl groups in starch and its derivatives.

Research under a PL 480 grant at the University of London, London, England, showed that pullulanase, the extracellular glycogen and amylopectin debranching enzyme of Aerobacter aerogenes is unusually resistant to heat inactivation. Its activity is totally destroyed by heat above 70° C. However, a considerable portion of this activity can be regained on storage at room temperature. The extent of reactivation depends on pH, enzyme concentration, and the specific enzyme preparation. The last variable appears to be the result of at least two factors. First, pullulanase preparations contain both a high and low molecular weight species. The presence of the former is necessary for the stabilization of the latter. Second, the preparations also contain a dialyzable glycoprotein ($M = 6,000-10,000$) which is necessary for stabilization of the enzyme. The proportion of these species in different preparations is variable. This research has been completed.

2. Structure and properties of polysaccharides. A highly sensitive micro-technique has revealed further evidence of the composition of polysaccharide Y-6272. Analysis of a length-of-hydrolysis series for rate of appearance of intermediate and final products revealed (1) N-acetyl-glucosamine as the initial product with rapid conversion to glucosamine, and (2) N-acetyl-hexosaminuronic acid and a disaccharide as intermediates to final products which appear to be hexosaminuronic acid and glucosamine. Substituent groups in polysaccharides from Arthrobacter strains B-1797 and B-3225 have been identified as: pyruvic acid 5.5%, and Q-acetyl 7.8% for B-1797; and pyruvic acid 5.1%, Q-acetyl 5-6% (tentative), and Q-succinyl 2-3% (tentative) for B-3225. Reinvestigation of Y-1842 phosphomannan structure uncovered two new components: a lightly phosphorylated polymannoside (mannose/P = 29) and a tetrasaccharide monophosphate. The α -mannan component (mannose/P = 29; $[\alpha]_D + 61^\circ$) appears to consist largely of 1,2-linked units. An improved procedure was devised for isolation of the mannosyl glucose end group from Y-2023 phosphomannan. The Y-2023 polymer was found to contain 2-Q-(α -D-mannosyl)-D-glucose, a new disaccharide.

Investigations showed that polysaccharide Y-2448 behaves in 1 M KCl like a high-molecular-weight polyelectrolyte ($M_w 20 \times 10^6$) whose conformation closely resembles that of a random coil. Viscosity studies of polysaccharide B-1459 in 4 M urea and 0.01 M ammonium acetate indicate high intrinsic viscosity. From incomplete sedimentation-diffusion studies, the molecular weight presently is estimated to be between 6×10^5 and 2×10^6 .

At the University of Wisconsin, Madison, Wisconsin, grant research showed that the degree of carbanilation of polysaccharides, as measured by incorporation of phenyl groups, could be readily determined by the use of ^{14}C -labeled phenyl isocyanate. Standard deviation is about 1 percent. The carbanilylated polysaccharide was completely deacetylated and depolymerized by treatment with 1.3 N HCl in dry 2-methoxyethanol at 56-59° for 6.5 hours. Methylation of the free acid form of polysaccharide B-1973 proceeded to 95 percent of theoretical in one step.

Under a PL 480 grant to the National Sugar Institute, Kanpur, India, scaled-up, preparative isolation (1 to 3 gram amounts) of several aldobiuronic acids and of an aldotriuronic acid has been achieved. Additional materials made ready for further use are a purified polysaccharide and the mixed barium salts of two additional polysaccharides.

B. Chemical and Physical Investigations to Improve Products

1. Physical properties of starch dispersions. At the University of Graz, Graz, Austria, most of the measuring techniques and theoretical basis required for obtaining valid rheological data on concentrated (order of 5 percent) starch-based dispersions has been established. "Diagrams of state" have been prepared that graphically relate viscosity to both time dependent effects (thixotropy) and shear rate dependence. Dilute solution viscometry has been applied to characterize the starch materials in usual, absolute terms. Other properties being determined on pastes are gel strength and shear modulus (elasticity). Shear rates encountered in commercial uses of starch in coating formulations have been determined and will be used in experimental work. This study is being conducted under a PL 480 grant.

In this phase of work under a PL 480 grant at the Slovenian Academy of Sciences and Arts, Ljubljana, Yugoslavia, corn, wheat, and sorghum starches of American origin were modified by a variety of physical and chemical treatments designed to effect changes in their physical properties without destroying the granule structure in order to impart to the starches new and useful paste properties. Treatment of American starches with moisture contents of 10-23 percent by heating at 100°-120° C. for 3-5 hours or at 55° C. for 48 hours with 0.1 N mineral acids or sodium phosphate generally produced the greatest changes in their gelatinization temperature, paste viscosity, setback, solubility, swelling power, and transparency. However, for corn starch, modification both with dilute acid or sodium phosphate and with ultrasonics (1,600 KHZ; 3w/cm²) for 2 minutes was needed to produce the greatest change in paste solubility, swelling power, and transparency. Some detectable differences were found between X-ray patterns of the unmodified starches and those modified with acid or heat. All modifications were carried out without significant change in granule structure of the starch.

2. New derivatives of starch and related carbohydrates. The crystalline solid recovered from the reaction of bromoacetaldehyde diethyl acetal with D-mannitol was determined to be a 2:1 mixture of cis-1,2:cis-5,6-di-O-(bromoethylidene)-D-mannitol and cis-1,2:trans-5,6-di-O-(bromoethylidene)-D-mannitol. Studies on 1,2-O-bromoethylidene-D-mannitol established that the mono bromoethylidene acetal formation was equilibrium controlled and that conversion to the dibromoethylidene acetal was kinetically controlled and irreversible. Acetals formed irreversibly are determined by the stereochemistry of the transition state and relative rate of formation. As a result, they may not conform to the Hahn-Hudson rules.

Studies showed that the cyclic carbonate group formed from vicinal trans hydroxyl groups in pyranoid rings readily undergoes ring opening with various simple nucleophiles. The trans-fused cyclic carbonate and thiocarbonate groups also react with the hydroxyl groups of starch under various conditions to yield a starch product with pendent carbohydrate residues. An easy route was established for covalently bonding amino acids to carbohydrates, and conditions were found for the polymerization of carbohydrate trans-carbonates.

Grant research at the Ohio State Research Foundation, Columbus, Ohio, showed that reaction of dialdehyde starch and nitromethane yielded the expected product formed by addition of nitromethane to the aldehyde groups. Reduction of the nitro groups in this product to amino groups has not yet been achieved. Conditions were defined for the reaction of amylose with sulfuryl chloride to obtain a product completely chlorinated at C-6 and containing a cyclic sulfate. This product was subjected to hydrazinolysis, reduction with Raney nickel, and dialysis to yield an aminated amylose of D.S. 1 which had a low sulfur content (0.2%).

A synthetic method for preparing p-di(epoxyethyl)benzene has been developed under a grant to the University of Arizona, Tucson, Arizona. It involves reduction of p-di(chloroacetyl)benzene to p-di(α -hydroxyl- β -chloroethyl)-benzene with subsequent treatment with ethanolic potassium hydroxide. Studies show that diepoxides and polyols react to yield polymers which are probably crosslinked heteropolymers. Both m- and p-di(epoxyethyl)benzene have been synthesized to study effects of positional isomerism on the diepoxide-polyol polymerization. Synthesis of the ortho isomer proved to be impractical. This work is part of a study of the formation of polymers by reaction of diepoxides with starch and related carbohydrates.

Under grant research at Purdue University, Lafayette, Indiana, phosphine and phenylphosphine were added to the terminal olefinic bond of 5,6-dideoxy-1,2-O-isopropylidene- α -D-xylo-hex-5-enofuranose under ultraviolet radiation. Two convenient routes for introducing nitrogen at carbon five of D-glucose, beginning with 5-O-tosyl derivatives of 1,2-isopropylidene- α -D-glucofuranose, were found. These reactions have led to D-glucose derivatives with phosphorus or nitrogen replacing the pyranose ring oxygen, thus providing properties totally different from those of normal sugar.

In studies under a grant to the Ohio State Research Foundation, Columbus, Ohio, acetylated 1-thioaldopyranoses having the D-gluco, D-galacto, D-xylo, D-ribo, and D- and L-arabino configurations were synthesized and characterized for work on glycosulphenyl halides which are derived by halogenation of the thio sugar derivatives. New cyclopropyl derivatives, prepared from unsaturated sugars, were characterized. An easy method for preparing dimethylthiocarbamates, useful in simplifying NMR spectra, was demonstrated. These derivatives also offer a potential route to thio sugars by thermolysis or photolysis. This grant research project was completed and a final report has been received.

At Southern Illinois University, Carbondale, Illinois, under a grant, the effects of temperature and of different alcohols upon the hydrogen chloride-catalyzed alcoholysis of amylose triacetate and D-glucose pentaacetate to form alkyl glucosides were investigated. Most of the alcoholyses produced 3:1 to 4:1 ratios of α - to β -glucosides at 30° C., 55° C., or at reflux. A synthetic route to alkyl α -glucoside was provided by the direct removal of β -glucoside with an anion-exchange resin. A homologous series of methyl terminal 4-deoxymaltooligosaccharides was prepared. A terminal 4-deoxy group did not block β -amylase action, whereas terminal 4-methoxy did. Contrary to indications in the literature, participation of a neighboring group is not advantageous in 4-O-tosyl or 4-O-methyl displacements.

In studies under a PL 480 grant at the Institute for Fibres and Forest Products Research, Jerusalem, Israel, the oxidation of wheat starch and waxy maize starch by bromine in acid aqueous medium was found to be a first order reaction. Pure amylose was unaffected by bromine at pH 3 and 1.7, probably because of the formation of the bromine-amylose complex.

Through extensive studies with model compounds at the Plastics Research Institute TNO, Delft, The Netherlands, a highly satisfactory method was developed for determining the position or positions occupied by the metal groups in metal starchates prepared under anhydrous conditions by previously reported procedures. The most suitable method of analysis was conversion of the metal starchates to the corresponding methyl ethers followed by hydrolysis with hydrochloric acid to glucose methyl ethers, which in turn were converted to trimethylsilyl derivatives and analyzed by gas-liquid chromatography. This work is being conducted under a PL 480 grant.

3. Starch graft copolymers. Viscosities of base-hydrolyzed starch-PAN (1:2) with an average grafted side chain (GSC) molecular weight of 780,000 (1 graft/2,400 AGU) in aqueous solution were the highest so far determined. Increasing the GSC molecular weight of PAN in starch-PAN from 286,000 to 780,000 increased the viscosity of the hydrolyzed products by 70 percent, as did increasing the grafting frequency of PAN from 4,623 to 2,435 AGU/graft when the GSC molecular weight was kept near 800,000. Starch-PAN graft copolymer (2:1) with a GSC molecular weight of 1,045,000 was prepared and hydrolyzed under a variety of conditions. Viscosities were up to 37 percent lower than those for 1:1 starch-PAN of GSC molecular weight of 800,000. Comparison of viscosities and salt tolerance of all hydrolyzed starch-PAN products $\overline{H(S-PAN)}$ indicated that H(S-PAN) (1:1), GSC molecular weight 800,000, and H(S-PAN) (1:2), GSC molecular weight 780,000 are the most promising as water thickeners. A study showed that ceric ammonium nitrate-initiated graft polymerization of PAN onto a number of modified starches resulted in less homogeneous products.

In research completed during the year under a PL 480 grant to the Hebrew University, Jerusalem, Israel, graft copolymers of starch and polymethyl methacrylate were prepared by treating preformed methyl methacrylate

polymers with starch alkoxide dissolved in DMSO. Although considerable grafting occurred (presumably via transesterification), the reaction products were quite inhomogeneous and not judged to have much potential utility. Exploratory studies showed that the chloroformyl ester derivative of ethylene oxide prepolymers reacts with starch alkoxide to form graft copolymers. Glycidyl methacrylate was grafted to starch by anionic means to give 55-67 percent weight yields of purified graft copolymer.

4. Chemical products from starch. Direct chlorination of low D.S. cyanoethylated starch under alkaline conditions produced products whose pastes have favorable viscosity properties and high clarity for potential use as a pigment coating adhesive. Paper coated with clay containing this type of adhesive had high wax pick values.

Resorcinol-formaldehyde-treated starch as a reinforcing agent in two types of styrene-butadiene-rubber (SBR), nitrile, and natural rubber has been studied in detail. In SBR 2105 the best tensile properties were achieved with 45 phr starch and 8 percent resorcinol-formaldehyde based on starch.

Survey studies were made to determine the utility of various other formaldehyde condensation products, in addition to resorcinol-formaldehyde, for treatment of starch to obtain reinforcing agents. Highly reactive phenol bases, pyrogallol and catechol were as effective as resorcinol-formaldehyde but are expensive. Low-cost bases were not reactive enough. A wide variety of resol-type resins, particularly phenol-formaldehyde and melamine-formaldehyde, could be employed to treat starch for improved reinforcement.

Resorcinol-formaldehyde also functioned as an additive to zinc starch xanthate, giving about double the reinforcement of the latter when used alone in paper. There was no interference with the xanthate crosslinking reaction in coprecipitation. Paper treated with starch polyethylenimino thiouran was studied to determine its ion exchange (acid and base) capacity; it was found to be stable over a short range of repeated cycles. Several polyamines that are low-molecular-weight analogs of polyethylenimine have been found to be gel formers in reaction with starch xanthate.

Urethane plastics, obtained by pressure-molding mixtures of starch, castor oil, and pp'-diphenylmethane diisocyanate, showed a decrease in tensile strength after 1 week of aging in 10-percent NaOH, 3 percent sulfuric acid, or hot water (65° C.). The use of modified polyols instead of castor oil increased tensile strength and resistance to hot water, alkali, and acid. The polyols were obtained by heating castor oil with glycol glycosides, sorbitol, or ethylene glycol in the presence of a catalyst.

Contract research completed during the year at the Institute of Paper Chemistry, Appleton, Wisconsin, showed that starch xanthides added to paper pulp furnishes are adsorbed by processes involving electrostatic interactions. Sorption activation energy of starch xanthide prepared from D.S. 0.08 starch xanthate was found to be nearly the same as that from D.S. 0.12 starch

xanthate. Pulp consistency had little effect upon xanthide sorption if the alum concentration was maintained between 2×10^{-4} and 2×10^{-5} M. Over this range, alum concentration influences xanthide retention and effectiveness more than does fiber surface area or the sorption temperature.

At the Ahmedabad Textile Industry's Research Association, Ahmedabad, India, the preparation of copolymers of cereal starches with polysaccharide gums such as guar, locust bean gum, and gum arabic was investigated. Mixtures of starch and these gums in several ratios were heated at various temperatures and times with different amounts of hydrochloric acid catalyst, and the products were isolated and characterized. The products obtained from starch and gum guar were evaluated at the Northern Division as deflocculant additives in papermaking. To date none of the products shows promise for this use. These studies, conducted under a PL 480 grant, were completed during the year.

In other research under a PL 480 grant to this institution, a commercial sample of hydroxyethylated corn starch of degree of substitution of 0.1 was characterized, and the distribution of hydroxyethyl groups in the anhydroglucose monomer unit of the starch was determined. The results indicated that the C-2 position of the anhydroglucose unit was etherified to the extent of 84 percent. Most of the remaining ether groups were located at the C-6 position with negligible reaction at C-3. These findings were corroborated by periodate oxidation. A new crystalline compound, anhydro mono-O-hydroxymethyl-D-glucose, was isolated and characterized.

5. High-amylose (HA) starch film. Viscose solutions, prepared and blended with various proportions of alkali-dispersed HA starch, produced films with good clarity and flexibility. However, values for tensile strength, percent elongation, and MIT fold test were lower than those for cellophane. Film prepared from HA starch acetate of low D.S. showed improved flexibility when starch granules surviving acetylation were disrupted. Gelatinization of the HA starch acetate in an autoclave appears to be the most practical method among several tested for obtaining a material that will give a more flexible film at low relative humidities.

6. Thermal reactions of starch. In studies under a grant to the University of Pittsburgh, Pittsburgh, Pennsylvania, when cornstarch was pretreated with a 1:1 acetone-water wash to limit foaming, levoglucosan was obtained in 43 percent crude yield in a batch process run in a tubular reactor with ordinary electric heating. Two continuous reactors with screw-type conveyors were designed for vacuum operation. The 1-inch diameter reactor with a combination of electric and dielectric field heating, gave 30 percent yields but clogging was encountered. A 4-inch reactor is being constructed with flexible agitator blades to alleviate the problem.

At the University of Edinburgh, Edinburgh, Scotland, investigations on thermal degradation of starches revealed the following results for

differential thermal analysis (DTA) of starches and maltodextrins:

(1) Simple salts markedly affect the thermograms; (2) differences in thermograms of common starches are small and hence DTA cannot be used to characterize various starches; (3) the initial endotherm for amylopectin was lower than that for amylose; (4) the physical structure of the starch granule had the greatest importance in determining thermogram properties; and (5) thermograms of maltodextrins are similar to those of starch, with thermal stability increasing with chain length of oligomer. This work is being conducted under a PL 480 grant.

C. Microbiology and Fermentation

1. ARS culture collection. During 1967, 1,377 molds, 598 yeasts, and 612 bacterial cultures were distributed. Of these, 2,044 were supplied to domestic investigators and 543 were sent abroad. Twenty-five named cultures of Alternaria were acquired from the Quartermaster Depot Laboratories and 22 from the American Type Culture Collection for use in the antibiotics screening program. Several samples of tall fescue were collected from a Missouri pasture in which cattle showed signs of fescue foot toxicity. A large number (201) of isolates of fungi were placed in the temporary collection for further examination. (See also "Forage Utilization - Northern Region" RPA 407.)

Satisfactory conditions for sporulation of breeding stocks of Hansenula holstii were established. Both temperature and composition of the sporulation medium were critical. Through the use of genetically marked strains, evidence was uncovered for the presence of two independent complementary sets of spore-lethal factors in the breeding stocks. Segregation of three auxotrophic markers were followed in two independently derived diploids. In each, segregation of the nutritional markers relative to one another and to viable and nonviable spores were inconsistent from one sporulation to another. These diploids exhibited unique morphological changes during vegetative growth. Numerous stalked cells bearing a terminal bud appeared in young populations. Segregation ratios of auxotrophic markers in clones derived from the terminal bud and from the parent cell of these stalked forms differed markedly. The locus controlling agglutinating cell types of H. wingei segregated with that determining mating type in each of 600 tetrads analyzed. Various intercrosses and backcrosses have been started to establish cell lines which consistently produce either strongly or weakly agglutinating progeny. Production of an extracellular agglutination inhibitor is apparently determined by a single genetic factor.

Contract research by the American Type Culture Collection, Rockville, Maryland, revealed that the 10⁴ strains of fungi previously reported have now survived a total of 30 months of storage at liquid nitrogen temperatures; 99 were suspended in 10 percent glycerol and 5 in 10 percent DMSO.

Initial grant research efforts at Baylor University, Houston, Texas, have been directed toward gaining an insight into organisms of the genus

Hansenula and toward developing techniques for detailed study of their cytology. Experiments have dealt with definition of growth of haploid and diploid strains under varying conditions, characterization of sporulation of diploid cells, and the correlated phase-contrast microscopy of both. Initial attempts to prepare cells for electron microscopy show promise.

Fungus and Streptomyces strains known to produce high yields of citric, fumaric, and gluconic acids; penicillin and streptomycin antibiotics; and proteolytic and amylolytic enzymes were investigated. A strain producing fat was also studied. For each microorganism, spores derived from both lyophilized and unlyophilized preparations were used to start appropriate fermentations. Statistical analysis and comparison of the results of hundreds of such fermentations indicated very definitely that lyophilization did not result in selection of lower yielding strains. Hence, lyophilization of industrially important cultures is a safe method to maintain strains without deterioration of their ability to form desirable products. This research, completed during the year, was conducted at the University of Allahabad, Allahabad, India, under a PL 480 grant.

In the past year 14 streptomycete cultures, with supporting taxonomic data for each, have been submitted by the Central Drug Research Institute, Lucknow, India, in accordance with the provisions of a PL 480 grant. All have been tested at the Northern Division for their ability to produce antibiotics. Eight of the cultures demonstrated activity against a gram-positive bacterium (Bacillus subtilis); five against a gram-negative plant pathogenic bacterium (Agrobacterium tumefaciens); and seven against a mold (Mucor ramannianus). In addition, one was particularly active against yeasts. All of the cultures have been added to the ARS Culture Collection for use in future screening programs.

Sixty streptomycete cultures, with taxonomic data and electron micrographs for each, have been submitted by the National Institute of Agronomic Research, Madrid, Spain, as provided by a PL 480 grant. A total of 33 of the cultures have been tested at the Northern Division for their ability to produce antibiotics. Activity was demonstrated by 12 against a gram-positive bacterium (Bacillus subtilis); by 3 against a plant pathogenic gram-negative bacterium (Agrobacterium tumefaciens); and by 11 against a mold (Mucor ramannianus). All of the cultures have been added to the ARS Culture Collection for use in future screening programs.

2. Studies on enzymes. Enzymic reactions are being investigated to evaluate possibilities of their use to prepare chemical products for industrial applications.

A routine method was developed to obtain glucose-6-phosphate dehydrogenase by stepwise elution chromatography on hydroxyapatite. Overall recovery is 23 percent with 30-fold purification. Glucaric acid dehydratase, purified

ninefold by precipitation with $(\text{NH}_4)_2\text{SO}_4$ and chromatographed on DEAE-cellulose columns, showed only 50 percent of its activity after being chromatographed. Transglucosyl amylase has been tentatively shown by electrophoresis and carbohydrate tests to be a glycoprotein. Gluco-amylase can be immobilized on DEAE-cellulose at pH 4.0 in 0.05 M sodium acetate. A dilute (1%) starch solution passed through a column with the suspended enzyme shows glucose formation equivalent to hydrolysis by the free enzyme. Apparent activity of the bound enzyme is about one-sixth that of the free enzyme.

By isolation of the corresponding peptide dimers after a series of enzymic degradations, two types of crosslinkages were demonstrated in Micrococcus lysodeikticus cell wall glycopeptide. In one, pentapeptide monomers were linked through N-(D-alanyl)-lysine. This link is hydrolyzed by Myxobacter ALI protease. Pentapeptide monomers of the second dimer were linked through the D-alanyl-L-alanine linkage, split by Streptomyces ML endopeptidase. A Streptomyces exo-N-acetyl hexosaminidase was found which acts as both an exo- and endo- β -N-acetylmuramidase.

Attention was turned to wall peptidoglycans which contain α, α' -diamino-pimelic acid (DAP) and the stereochemistry of linkages involving this component. In Bacillus megaterium KM, the meso isomer of DAP predominates and the amino groups on the L-carbons are involved in peptide linkages to glutamic acid residues. While most of the amino groups on the D-carbon are free, some are substituted and probably crosslink peptide subunits since they are liberated by a new Streptomyces endopeptidase. Minor amounts of D, D-DAP are present. These residues have no free amino groups; endopeptidase treatment does not liberate these groups. This research is conducted under a PL 480 grant to the University of Liege, Liege, Belgium.

3. Fermentation with spores. Three Aspergillus species, A. wentii, A. flavus, and A. oryzae, were shown to have strong invertase activity. Preliminary studies showed spores of A. oryzae capable of metabolizing galactose to three new metabolites whose identities are under investigation. Low levels of tetracycline added to spore suspensions effectively controlled bacterial contamination without interfering with metabolic activity. A new method of column fermentation was developed in which fungal spores were either trapped in a resinous matrix or crosslinked with a synthetic polymer. Substrate flow rate was affected by the ratio of spores to suspending matrix and the percent conversion by the concentration of substrate and column height. The same column was still active after standing for 3 weeks at room temperature.

4. Biological insecticides for Japanese beetle. A lytic enzyme, contained in culture supernates of a Bacillus sp., P-1167, was found to lyse vegetative cells of B. popilliae but not affect the sporangium of spores or pre-spores. Availability of this lytic enzyme affords the means for purifying spore preparations produced in vitro. Sporulation on solid media has been

increased to about 20-30 percent and standard conditions yield about 5×10^7 spores per ml. Sporulation in liquid medium containing carbon has been increased to 1.5×10^6 spores per ml. Effective sporulation in both solid and liquid media depends upon inocula prepared with minimum vegetative transfer from the spore stage. Work has been initiated to study the hemolymph lipids of healthy and diseased grubs. This is the only major component of hemolymph not as yet examined.

Contract studies at the University of Minnesota, Minneapolis, Minnesota, on the transfer of genetic determinants of sporulation have been completed. Final results indicate that four separate gene elements covering two proteolytic enzymes, an antibacterial factor, and competence are associated with the gene controlling spore formation in Bacillus subtilis. "Controller" genes also are indicated. Genetically controlled nutritional deficiencies also influence sporulation. Transformation of Bacillus cereus was not possible. Competence for uptake of extracellular DNA was not detected under any condition tested with a variety of DNA species including that from B. cereus phage. These phages were isolated and characterized. No evidence for an episomal DNA regulating sporulation was detected. Data indicated the presence of an inhibitory material in vegetative cultures of Bacillus popilliae which could be partially removed by dialysis or growth in a biphasic system. In addition, an unidentified nutrient was supplied by associated growth of a Rhodotorula yeast. Presporulation or early sporulation events occurred when B. popilliae was grown in association with Rhodotorula in a biphasic culture system.

In contract research at Baylor University, Houston, Texas, cells from hemolymph in early stages of infection were fixed immediately upon collection and examined by electron microscopy. Vegetative cells and prespores prepared in this manner are indistinguishable from vegetative cells of laboratory cultures. In confirmation of NU observations, a large proportion of vegetative cells at all stages of infection appear granular or light by phase microscopy and are largely devoid of cytoplasm by electron microscopy. Thus, most vegetative cells in the hemolymph apparently do not sporulate and die. The 20 percent maximum sporulation obtained in colonies may be about the same as that in the larvae and may be all that can be expected in an artificial culture system for producing spores. Research work under this contract has been completed.

Techniques, based on label distribution in metabolized products from ^{14}C substrates, have been developed under a contract at Michigan State University, East Lansing, Michigan, for following the biochemical changes which occur during growth and sporulation of sporogenic and asporogenic strains of Bacillus popilliae on solid media. Further study of the occurrence and properties of catalase, a heme enzyme, in spores and refractile bodies was undertaken. With NRRL B-2309M, addition of whole blood prior to autoclaving caused up to 36 percent sporulation on a solid medium. This finding is contrary to results of earlier studies by NU's Fermentation Laboratory with blood and blood components. Labeled barbiturate has been shown to be incorporated into cellular RNA, but the pathway is not known.

5. Preparation of microbial polysaccharides. Under a grant to Cornell University, Ithaca, New York, continued studies on cells of yeasts and bacteria coated with positively charged colloidal aluminum oxide show that the cells can be readily coagulated by addition of uncoated cells. The coagulation was more efficient than that obtained with cationic polymers ordinarily used for this purpose. The presence of phosphate has been found to be of critical importance in the flocculating process. This work is part of a study to find new fermentation techniques that could lead to increased yields in production of microbial polysaccharides.

6. Fermentation acids. Research on fermentation acids is in progress under PL 480 grants at several foreign institutions.

At the University of Lodz, Lodz, Poland, a comprehensive program of selection of mutants of Aspergillus terreus for superior strains with which to produce itatartaric acid has been completed. Some mutant strains do, indeed, produce considerably more itatartaric acid than the parent strains, but the yield is still too low to be commercially practical even though a thorough study was undertaken to find the best medium and physical environment for acid production.

It has been found that itatartaric acid exists in aqueous solution in equilibrium with β -hydroxyparaconic acid in nearly equal molar ratios. When this fact is taken into consideration, the actual yield of the itatartaric acid complex is nearly double the apparent analytical values.

At the University of Tokyo, Tokyo, Japan, five strains of Acetobacter suboxydans have been isolated that produce dextrorotatory tartaric acid from either glucose or 5-ketogluconic acid. A strain of Sporobolomyces yeast and a Brevibacterium strain were also found to produce dextrorotatory acid, but only from 5-ketogluconic acid. A study of cultural conditions of two strains of A. suboxydans succeeded in increasing yields of tartaric acid somewhat.

The pathway to tartaric acid appears to be from glucose, through gluconic, 5-ketogluconic and glycolic acids, to tartaric acid. 2-Ketogluconic acid is a byproduct not utilizable for tartaric acid formation. A program is underway to find mutants that are unable to produce 2-ketogluconic acid and would then presumably produce greater yields of tartaric acid.

In other research at the University of Tokyo, Endomycopsis fibuliger IAM 4347 (now NRRL Y-7069) was selected by extensive survey for production of mevalonic acid. Maximum yield was 1,000 μ g per ml. in 4-5 day aerobic fermentations at 30° C. The culture medium contained 10% glucose, 0.5% polypeptone, 0.1% yeast extract, 0.5% KH_2PO_4 , 0.3% NH_4Cl , 0.05% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 1% CaCO_3 . Although not required, calcium pantothenate often stimulated mevalonate production as did KCN added on the fourth day. In addition, two crystalline antibiotics (brefeldin A and varrucarin A) inhibitory to

animal viruses were isolated from soil fungi. The antibiotics are effective against Newcastle disease virus strain Miyadera, herpes simplex strain HF, and vaccinia virus. Plague-inhibition tests using chick embryo fibroblast cell monolayers and tube culture assays were used. Although these substances are known antibiotics, their antiviral activity has not been reported before. The chemotherapeutic index of brefeldin A using HeLa cells is approximately one and that of verrucarin A is two. This research was completed during the year.

7. Plant antibiotics. Chromatographic studies of the new antiyeast antibiotic produced by Alternaria oleracea show it to be different from known available antibiotics. Screening of 160 additional Alternaria strains uncovered 94 more species with antimicrobial activity. One of several antibiotics produced by A. brassicicola was isolated and partially characterized. Nuclear magnetic resonance and spectral studies indicated the presence of a hydroxyl, a nitrile, and several methylene groups as well as considerable unsaturation. The elemental formula was determined as $C_{25}H_{39}O_{11}N$ with a molecular weight of 540. It was optically active with a rotation $[\alpha]_D^{25} + 20.1$. Biological tests showed activity against some molds and bacteria but not against yeasts. It remained stable when autoclaved at 121° C. This project was discontinued during this reporting period.

8. Beta-carotene synthesis. Under a PL 480 grant to the Agricultural University, Poznan, Poland, the following factors that affect β -carotene production by mated cultures of Blakeslea trispora were studied:

(a) Replacement of citrus molasses by spent mycelium; (b) use of spent mycelium as an adjunct to the medium; (c) stimulatory activity of various microbial cells. Aqueous extracts of the mycelium and the organic acid fraction of these extracts did not enhance carotene yields. Various prepared digests of spent mycelium enhanced yields only to the same extent as untreated spent mycelium. Spent mycelia could partially replace both the cottonseed embryo meal and citrus molasses, thereby lowering the cost of the fermentation medium in the process.

D. Technology--Process and Product Development

1. Cereal xanthides and xanthates. Increasing the number of thionocarbonate groups in xanthide-treated paper failed to improve the wet strength of handsheets. A new laboratory method of incorporating xanthide was found which increased wet-tensile values for handsheets from 2,200-2,500 to 3,300-3,900 meters without increasing the amount of starch xanthide required. Aging tests on the process revealed that paper made with starch xanthide showed no significant loss in sulfur content during storage for 30 days.

The considerable quantities of data obtained in machine trials of cereal-derived products on the 32-inch paper machine are being processed by computer. A pilot-plant apparatus for continuous production and application

of starch xanthides has been constructed and used in evaluations of process variables. As wet-end additives, starch xanthate-polyethylenimine reaction products are equal to or better than commercial cationic starches of high quality and, in addition, provide wet strength.

2. Starch derivatives for protective coatings. In contract research at Battelle Memorial Institute, Columbus, Ohio, exposure of the exterior paint series for 18 months indicates some mildew growth, particularly on those paints containing dibutyl tin dilaurate. Some failure by grain cracking has occurred but no significant changes due to the use of starch derivatives have resulted. Interior exposures to north light over a 15-month period have deteriorated only to the point where discoloration is just barely visible to the naked eye. With potassium carboxymethyl starch as an additive, yellowing of the styrene-butadiene-latex paint appeared to be decreased. This result, if confirmed, could have industrial importance. Starch derivatives appear to soften interior paint films, a condition which could probably be corrected by including a small amount of zinc oxide in the formula.

Alcoholysis products were successfully prepared from dehydrated castor oil and safflower oil under a research contract at Archer Daniels Midland Company. Alkyds satisfactory for evaluation were achieved by the reaction of safflower oil with tetrahydrophthalic anhydride but not with castor oil. Hypophosphorus acid was an excellent color retardant for both alkyd and glycoside synthesis. Drying time for alkyds, which is increased by hypophosphorus acid, has been reduced to acceptable levels by decreasing the amount of hypophosphorus acid and increasing the amount of lead naphthenate drier. These modifications permitted satisfactory preparation of glycoside-based alkyds with long, medium, and short oil lengths from soybean, linseed, and safflower oils.

3. Protein-dialdehyde starch (DAS) glue for southern pine plywood. An exploratory mill evaluation of protein-DAS plywood glue was made under conditions more rigorous than normal owing to 39° F. ambient mill temperature at time of trial. Lower grade veneers were substituted for those scheduled because of mill problems with veneer drying. Thirty-four standard panels were prepared using typical production schedules. Quality glue bond tests were conducted by the American Plywood Association and resulted in a maximum rating of 62. A rating of 90 is required for a candidate glue subject to an approval test and an approved glue must maintain a rating of 85 in commercial production. Low moisture content in some of the veneers was indicated as responsible for erratic test results observed in this evaluation of the protein-DAS glue. Low-moisture veneers are responsible for major gluing problems encountered by the southern pine plywood industry, which currently employs phenolic resin glue.

4. Graft copolymers. Contract research on starch-acrylamide (AA), starch-acrylic (AAC), and starch acrylonitrile (AN) grafts at General Mills, Minneapolis, Minnesota, has shown all of the graft copolymers except AN to

have greater water solubility and lower gelatinization temperatures than ungrafted starch. All of the graft copolymers effectively thicken 15-percent sodium hydroxide solutions and retain good viscosity during storage for several months. In HCl only AA grafts hydrate and show potential as thickeners. None hydrate or disperse in an electrolyte such as 10 or 25 percent NaCl. Fractionation studies showed all products from AA or AN grafts to be heterogeneous. AN from pilot preparation appeared to contain a large amount of ungrafted starch as compared to that prepared in the laboratory. Upon alkaline hydrolysis, however, both formed extremely viscous solutions indicating that composition and molecular weight may vary considerably without producing gross changes in the properties of the hydrolysis product.

5. Starch for rubber reinforcement. Under a research contract, scientists at the University of Akron, Akron, Ohio, prepared SBR masterbatches containing up to 100 phr of starch xanthide incorporated by oxidative coupling with sodium nitrite. Starch xanthide accelerated vulcanization, gave large increases in rubber modulus, and reinforced more effectively than zinc starch xanthate. A variety of starch xanthate-rubber coprecipitation procedures have been developed for practical production of starch reinforced masterbatches.

6. Microbial polysaccharides. In studies on continuous fermentation, a 25-gal. single-stage fermentation for production of B-1459 was run for 13 days. Steady state conditions were maintained for 10 days, during which the daily yield was 81 percent based on glucose. The overall yield was about 75 percent. Fermentation cost of this continuous process, including further improvements in medium, is estimated at 16 cents vs. 19 cents per pound for the 48-hour batch fermentation.

Studies on kinetics of batch fermentation enabled reduction in fermentation time from 48 to 32 hours. Fixed capital investment is reduced by 20 percent and cost of product is decreased by 1.2 cents per pound.

RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS AND NATURALLY OCCURRING TOXINS

C. Microbiology and Toxicology

1. Aflatoxin investigations. Numerous organisms were examined for a fungal system capable of catalyzing aflatoxin transformations. Several fungi are capable of transforming about 50-60 percent of aflatoxin B₁ into a compound having 1/20th as much toxicity, but none was found which was capable of total degradation of aflatoxin. A fluorescing compound separated by silica gel chromatography from an aflatoxin M sample could be aspertoxin. This new mycotoxin was recently reported to have been found in Aspergillus flavus. Cultures capable of degrading coumarin are being further screened for mutants with the ability to degrade aflatoxin.

In contract research at the University of South Dakota, Brookings, South Dakota, Aspergillus ochraceus and A. sulphureus strains were grown on soybeans and wheat and checked for toxicity on chicks and mice. The A. ochraceus group has the largest number of strains to date which produce mycotoxins on wheat and soybeans. Many toxic strains were found in the A. fumigatus group, but deaths in toxicity tests on mice were attributed to lung abscesses, congestion, pneumonia, and enteritis rather than to liver damage. Low toxicity was found in the A. niger group and the A. restrictus group.

2. Reduction of viable microorganisms in corn dry-milled products. Highest microbial populations in corn dry-milled products were in the flour, degerminator fines, and feed fractions. Three methods for effective microbial control are (1) treating corn before processing by steaming for 1 minute, heating at 200° F. for 20 minutes, or treating with hot solutions of sanitizing agents; (2) heating the degerminator streams at 150° F. for 1 hour at 15-20 percent moisture before milling; or (3) treating the dry-milled product after milling at 200° F. temperature for 10 minutes.

3. Microbiological processes and products. The Pioneering Laboratory for Microbiological Chemistry conducts a long-term program of basic investigations on the metabolic processes of microorganisms and on the metabolites they elaborate. In studies on linkages in the cell walls of spiral and rod-shaped bacteria, spiral walls were found to be the more extensively cross-linked. The regulation of lipid methylations into N-methyl, cyclopropane and methyl-branched groups is being investigated with methionine mutants of Agrobacterium tumefaciens. Zymonic acid ($C_6H_6O_5$), first isolated at the Northern Division in 1950, has been shown to be produced by 16 species of yeasts from 13 different genera. Eleven crystalline azo derivatives of zymonic acid have been prepared and their chemistry has been investigated. An unidentified yeast (NRRL Y-6954) has been found to excrete 3-D-hydroxypalmitic acid in copious amounts. The substance, occurring on cell walls of one mating type of a yeast, which causes sexual agglutination with the other mating type, was studied. Each active site in this substance is a protein, stabilized by one disulfide bond. Structures of no other sexually agglutinative substances are known in this much detail.

RPA 901 - ALLEVIATE SOIL, WATER AND AIR POLLUTION

B. Chemical and Physical Investigations to Improve Products

1. Biodegradable surfactants and detergent builders. A series of unsaturated di- and tricarboxylic acids were reacted with hypochlorous acid and epoxidized with alkali. These epoxy intermediates failed to react appreciably with starch in granule form. The chlorohydrin of aconitic acid readily hydrolyzes to dihydroxytricarballic acid. This compound is a good sequestering agent for calcium and magnesium in alkaline solution and will be studied as a builder for detergents.

These studies are supplemented by a contract with Ashland Chemical Company, Minneapolis, Minnesota, for research on surfactants from polyol glycosides and by a grant to Western Michigan University, Kalamazoo, Michigan, for investigation of homogeneous catalytic oxidation of starch. Since this work has only recently been initiated by these institutions, significant results have not yet been obtained.

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RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

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RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Industrial Products

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RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS
AND NATURALLY OCCURRING TOXINS

Microbiology and Toxicology

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WHEAT UTILIZATION (NORTHERN REGION)

USDA and Cooperative Programs

Location and Nature of Intramural Work	:Scientist Man-Years FY 1968:				
	: Research Problem Area				Total
	: 406	: 407	: 702	: 901	:
Peoria, Illinois	:	:	:	:	:
	:	:	:	:	:
<u>Food</u>	:	:	:	:	:
A. Chemical Composition and Physical Properties	:	:	:	:	:
	: 8.0	:	:	:	: 8.0
B. Microbiology and Toxicology	: 0.7	:	: 6.3	:	: 7.0
C. Technology--Process and Product Development	:	:	:	:	:
	: 1.5	:	:	:	: 1.5
	:	:	:	:	:
	:	:	:	:	:
<u>Industrial Products</u>	:	:	:	:	:
A. Chemical Composition, Physi- cal Properties and Structure	:	:	:	:	:
	:	: 1.1	:	:	: 1.1
B. Chemical and Physical Investi- gations to Improve Products	:	:	:	:	:
	:	: 13.7	:	: 1.4	: 15.1
C. Microbiology and Fermentation	:	: 6.4	:	:	: 6.4
D. Technology--Process and Product Development	:	:	:	:	:
	:	: 4.5	:	:	: 4.5
Total	: 10.2	: 25.7	: 6.3	: 1.4	: 43.6

Intramural program is supplemented by extramural support representing (a) 0.5 SMY's at State Agricultural Experiment Stations^{1/}, and (b) 11.4 SMY's at other U. S. institutions^{2/}.

- ^{1/} RPA 407: 0.2 (Chemical Composition, Physical Properties and Structure)
RPA 702: 0.3 (Microbiology and Toxicology)
- ^{2/} RPA 406: 2.0 (Microbiology and Toxicology)
RPA 407: 8.5 (Chemical Composition, Physical Properties and Structure, 1.9; Chemical and Physical Investigations to Improve Products, 2.7; Microbiology and Fermentation, 1.3; Technology--Process and Product Development, 2.6)
RPA 702: 0.2 (Microbiology and Toxicology)
RPA 901: 0.7 (Chemical and Physical Investigations to Improve Products)

Problems and Objectives

The dominant factor in the wheat economy of the United States continues to be a production capacity that can outpace U.S. and foreign demand. Research on wheat seeks to solve the problems hindering the development of markets for the full productive capacity of U.S. agriculture. The Northern Division research seeks to find new industrial markets for wheat, particularly in the Pacific Northwest where corn is not grown, and to improve yield and quality of wheat flour for use in foods.

Major objectives of current research are to develop and evaluate alternate ways to:

1. Convert wheat flour and starch to chemical products whose use will be economically advantageous to selected segments of the paper industry.
2. Improve yield and quality of wheat milling fractions.

Progress - USDA and Cooperative Programs

RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

A. Chemical Composition and Physical Properties

1. Microscopic and ultrastructure of wheat. Protein films, formed on water surfaces and stained with osmic acid, show small lipid bodies which range in size from 0.25 μ to 0.025 μ . The abundance of these lipid bodies suggests that they may have originated from the oil of the aleurone cells or the germ and were introduced during milling. Electron microscope observations on patterns of the spread protein have not revealed any significant differences between good and poor quality hard and soft wheats. Small particles were observed which form beaded strands when protein is fixed and stained with uranyl acetate. Their size, about 300 Å in diameter, can be related to the molecular weight of native gluten and suggests a value in the neighborhood of 5-8 million.

2. Characterization of wheat gluten proteins. Comparison of glutenins from different varieties of wheat showed that proteins from better quality varieties are precipitated from solution by salt more easily than proteins from poorer quality wheats in the same class. The salt precipitation technique, which permits comparison of both type and amount of protein, may be useful in determinations of gluten quality on small amounts of flour. Starch gel electrophoresis of reduced and alkylated glutenins disclosed some differences among varieties representing different classes but there are few distinct differences among good and poor quality varieties in the same class. Carbohydrate content in the glutenins ranged from 3 to 18 percent, with hard wheats generally containing less than soft wheats. The solubility of glutenin proteins was improved by reduction and

S-aminoethylation. Fractional precipitation of aminoethylglutenin produced material especially rich in glycine and tyrosine and poor in histidine, methionine, valine, and cysteine. This material, which accounts for 22 percent of glutenin has a larger molecular volume than purified gliadin. A quantity of purified gliadin sufficient for an extended study on conformation was prepared from Ponca wheat flour.

3. Milling characteristics. Contract studies, completed during the year at the University of Nebraska, Lincoln, Nebraska, show that milling properties and protein content of wheat can be controlled by (1) crossing soft, high-protein wheats with hard; low-protein wheats and selecting crosses having desired qualities, and (2) treatment of wheat with enzymes having specificity for pectin or protein. Starch gel electrophoresis of reduced and alkylated glutenin fractions indicated qualitative differences among gluten proteins of 10 different varieties of wheat. Differences were greatest among varieties representing different classes and least among varieties in the same class. Ten distinct differences were apparent in glutenins from good and poor qualities in the same class. This result suggests that small structural differences in glutenins may produce substantial changes in quality.

4. Other relevant research. Research on wheat starch is integrated with that on corn starch. Results are reported under "Corn Utilization" RPA 406, Section A.

B. Microbiology and Toxicology

1. Rennin-like enzyme. The rennin-like enzyme produced by R. oligosporus was found to be stable at 40° C. or below and at pH of about 6. At pH below 2 or above 7, its activity was rapidly destroyed. When R. oligosporus was grown in a milk medium, the organism produced a compound which inhibited growth of the cheese-starting culture. The inhibitor is presumably a glycoprotein and can be separated from the milk-clotting enzymes by gel filtration.

2. Other relevant research. Research on wheat is integrated with similar studies on corn. Results are reported under "Corn Utilization," RPA 406, Section C.

C. Technology--Process and Product Development

1. Milling and fractionation. Pressure treatment of hard red winter wheat grains at three levels of elevated moistures prior to standard milling increased break flour yield, decreased reduction flour yield, and lowered the amount of coarse residue left in fractionation by air classification. It increased the protein content and reduced the ash content of patent flour. Caustic peeling of hard red winter wheats resulted in reduced response to milling and air-classification procedures. Yields of the finer fractions were lower with a corresponding increase in coarse residue.

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Industrial Products

A. Chemical Composition, Physical Properties and Structure

1. Chemistry of glycoprotein linkages. In grant research at Marquette University, Milwaukee, Wisconsin, novel approaches to the synthesis of glycosides of hydroxyamino acids were successful. The production of DL serine-O-xyloside in 30-percent yield from 2-nitroethanol xyloside indicates an economical route to large-scale syntheses of serine-O-glycosides. An O-xyloside of serine has been synthesized in 30-percent yield by the condensation of N-benzyloxycarbonylserine p-nitrobenzyl ester with 2,3,4,-tri-O-acetyl-D-xylosylbromide, followed by hydrogenation. The kinetics of hydrolysis of serine, threonine, and hydroxyproline-O-glycosides in acidic and alkaline solutions has been elaborated.

2. Fluidization of flour. Experiments with stirred fluidized beds, conducted at Iowa State University, Ames, Iowa, under a grant, revealed that the final percentage of expansion was greater for deeper beds. Comparisons of flours containing a fluidizing agent with starch and some other industrial powders demonstrated that flours respond moderately well to stirring in a fluidized bed. An equation has been developed for predicting diffusion rates of water in potato starch, giving an insight into its physical structure. The thermal conductivity of starch was found to be too high to be a limiting factor in most heat-exchange situations.

3. Other relevant research. Research on wheat starch is integrated with that on corn starch. Results are reported under "Corn Utilization," RPA 407, Section A.

B. Chemical and Physical Investigations to Improve Products

1. Chemical modification of wheat gluten. Reduction of disulfide bonds of wheat gluten and other proteins by NaH in DMSO requires at least 2 moles of NaH, and sulfinylmethyl carbanion apparently does not reduce sulfide bonds. Preliminary photochemical studies with cereal proteins indicates that radiation cleaves disulfide bonds and modifies aromatic amino acids. Labeling studies with radioactive DMSO conclusively prove that photolysis in DMSO results in incorporation of DMSO into the protein backbone. Halogenated acetic acids were found to add to alkyl vinyl ketones. This appears to be a novel organic chemistry reaction having potential synthetic utility.

Under grant research at the University of Chicago, Chicago, Illinois, a number of strained, highly reactive cyclic esters containing sulfur and phosphorus were synthesized. These include 2-hydroxy- α -toluenesulfonic acid sultone, the corresponding 5-nitro sultone, 4-nitro-catechol cyclic sulfate, and catechol cyclic phosphate. Kinetic studies on the reaction of

imidazole and N-methyl-imidazole with 2-hydroxy-5-nitro- α -toluenesulfonic acid sultone in deuterated aqueous solutions indicate that the imidazole nitrogen acts as a general base in these solvent systems. In contrast, the imidazoles participate in nucleophilic displacement reactions with the sultones in nonaqueous solvents such as dimethyl sulfoxide.

2. Other relevant research. Research on wheat starch is integrated with that on corn starch. Results are reported under "Corn Utilization," RPA 407, Section B.

C. Microbiology and Fermentation

D. Technology--Process and Product Development

Research in these categories is integrated with similar investigations on corn. Results are reported under "Corn Utilization," RPA 407, Sections C and D.

RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS
AND NATURALLY OCCURRING TOXINS

B. Microbiology and Toxicology

1. Reduction of viable microorganisms in flour. Strains of actinomycetes (552) and bacteria (145) isolated from wheat flour have been lyophilized and added to earlier collections to form a basis for future classification and fermentation studies on flour-inhabiting microorganisms.

2. Other relevant research. Research on aflatoxins and related studies pertinent to wheat utilization are integrated with similar studies on corn. Results are reported under "Corn Utilization," RPA 702.

RPA 901 - ALLEVIATE SOIL, WATER AND AIR POLLUTION

B. Chemical and Physical Investigations to Improve Products

Research in this category on wheat is integrated with similar studies on corn. Results are reported under "Corn Utilization," RPA 901.

Publications - USDA and Cooperative Programs

RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

Chemical Composition and Physical Properties

Seckinger, H. L., and Wolf, M. J. 1967. Lipid distribution in the protein matrix of wheat endosperm as observed by electron microscopy. Cereal Chem. 44(6), pp. 669-674.

Microbiology and Toxicology

Hesseltine, C. W., and Wang, H. L. 1967. Traditional fermented foods. Biotechnol. Bioeng. 9(3), pp. 275-288.

Technology--Process and Product Development

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RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Industrial Products

Chemical Composition, Physical Properties and Structure

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Wall, J. S. 1967. Origin and behavior of flour proteins. Baker's Dig. 41(5), pp. 36-42, 44.

Chemical and Physical Investigations to Improve Products

Cavins, J. F., and Friedman, M. 1967. New amino acids derived from reactions of ϵ -amino groups in proteins with α,β -unsaturated compounds. Biochemistry 6(12), pp. 3766-3770.

Cavins, J. F., and Friedman, M. 1968. Automatic integration and computation of amino acid analyses. Cereal Chem. 45(2), pp. 172-176.

Cavins, J. F., and Friedman, M. 1968. Specific modification of protein sulphydryl groups with α,β -unsaturated compounds. J. Biol. Chem. 243(12), pp. 3357-3360.

Friedman, M. 1967. Solvent effects in reactions of amino groups in amino acids, peptides, and proteins with α,β -unsaturated compounds. J. Amer. Chem. Soc. 89(18), pp. 4709-4713.

Friedman, M. 1967. Mechanism of the ninhydrin reaction. II. Preparation and spectral properties of reaction products from primary aromatic amines and ninhydrin hydrate. Can. J. Chem. 45(19), pp. 2271-2275.

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Krull, L. H., and Friedman, M. 1967. Reduction of protein disulfide bonds by sodium hydride in dimethyl sulfoxide. Biochem. Biophys. Res. Commun. 29(3), pp. 373-377.

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RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS
AND NATURALLY OCCURRING TOXINS

Microbiology and Toxicology

Hesseltine, C. W. 1968. Flour and wheat: Research on their microbiological flora. Baker's Dig. 42(3), pp. 40-42, 66.

Hesseltine, C. W. 1968. Microbiological research on wheat and flour. Proc. Symp. Infestation and Microbiological Control of Cereals and Cereal Products, sponsored by Central States Section, Amer. Ass. Cereal Chem., Feb. 16-17, 1968, St. Louis, Mo., 24 pp.

Pfeifer, V., and Vojnovich, C. 1968. Reducing the microbial population of wheat and wheat flour. Bulletin--Association of Operative Millers, Jan. 1968, pp. 3022-3024.

GRAIN SORGHUM UTILIZATION

USDA and Cooperative Programs

Location and Nature of Intramural Work	:Scientist Man Years FY 1968:					Total
	: Research Problem Area					
	: 406	: 407	: 702	: 901	:	
Peoria, Illinois	:	:	:	:	:	:
<u>Food</u>	:	:	:	:	:	:
A. Chemical Composition and Physical Properties	: 2.9	:	:	:	:	: 2.9
B. Microbiology and Toxicology	: 0.2	:	: 1.3	:	:	: 1.5
C. Technology--Process and Product Development	: 5.1	:	:	:	:	: 5.1
	:	:	:	:	:	:
	:	:	:	:	:	:
<u>Industrial Products</u>	:	:	:	:	:	:
A. Chemical Composition, Physi- cal Properties and Structure	:	: 0.3	:	:	:	: 0.3
B. Chemical and Physical Investi- gations to Improve Products	:	: 2.4	:	: 0.4	:	: 2.8
C. Microbiology and Fermentation	:	: 1.8	:	:	:	: 1.8
D. Technology--Process and Product Development	:	: 1.1	:	:	:	: 1.1
Total	: 8.2	: 5.6	: 1.3	: 0.4	:	: 15.5

Intramural program is supplemented by extramural support representing (a) 0.2 SMY's at State Agricultural Experiment Stations^{1/}, (b) 3.1 SMY's at other U. S. institutions^{2/}, and (c) P.L. 480 funds in 1 country representing 36,552 U. S. dollars equivalent.

^{1/} RPA 407: 0.1 (Chemical and Physical Investigations to Improve Products)
RPA 702: 0.1 (Microbiology and Toxicology)

^{2/} RPA 406: 1.4 (Chemical Composition and Physical Properties, 0.9;
Microbiology and Toxicology, 0.5)
RPA 407: 1.5 (Chemical and Physical Investigations to Improve
Products, 0.6; Microbiology and Fermentation, 0.2;
Technology--Process and Product Development, 0.7)
RPA 901: 0.2 (Chemical and Physical Investigations to Improve Products)

Problems and Objectives

The growing importance of grain sorghum as a cash crop and the increasing magnitude of production challenge technology to establish a pattern of utilization that will maintain or increase the economic value of the crop. Because the bulk of the U. S. crop is grown in a relatively limited region west of the Mississippi River, there are geographic areas where freight transportation advantages should permit increased food and industrial usage of grain sorghum without significant displacement of other cereal grains. Food usage, now taking only about 1 percent of production, could be increased by improving quality, acceptability, and suitability of grain sorghum products for food. Limited industrial outlets for grain sorghum already exist. Processing techniques specifically adapted to grain sorghum should result in improved products that can maintain present industrial outlets against competition from synthetics as well as lead to increased industrial utilization.

Major objectives of current research are to develop and evaluate alternate ways to:

1. Get needed data on composition and processing treatments for use in making new food products from grain sorghum.
2. Convert grain sorghum to new or improved industrial products via technology suited to the specific characteristics of this grain.

Progress - USDA and Cooperative Programs

RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

A. Chemical Composition and Physical Properties

1. Chemical and physical studies on sorghum proteins. Fifteen pounds of the sorghum hybrid RS610 was debranned and degermed with a Strong-Scott barley pearler. The grits were Buhler milled to produce 65 percent first and 35 percent short flours which were defatted with n-butanol. Of many solvents tested for extracting flour protein, 70 percent ethanol solution at 60° C. was best; it extracted 40 percent of the flour protein. After milling, three hybrids (OK612, RS626, and TE77) were extracted with 60 percent t-butanol at room temperature and with 60 percent ethanol at 60° C. Either extraction removed about 40 percent of the flour protein. The t-butanol extraction was preferred because it could be freeze-dried to yield a protein soluble in electrophoretic buffer whereas ethanol solubles required dialyzing against water to obtain a difficultly soluble precipitate.

In contract research at Kansas State University, Manhattan, Kansas, six chick feeding experiments involving amino acid supplementation were completed during this period. Results indicated that both methionine and

lysine are limiting and that the amino acids in grain sorghum are readily available. Rat-feeding studies on grain sorghum showed a direct relationship between lysine content of the diets and weight gains and protein efficiency. During the 5th and 6th weeks of the feeding trials all diets were supplemented with lysine to 100 percent of the National Research Council requirement. All diets produced equal weight gains during this period with the exception of one which was low in overall protein.

2. Other relevant research. Research on sorghum starch is integrated with that on corn starch. Results are reported under "Corn Utilization," RPA 406, Section A.

B. Microbiology and Toxicology

Research on grain sorghum is integrated with related studies on corn. Results are reported under "Corn Utilization," RPA 406, Section C.

C. Technology--Process and Product Development

1. Processing studies. In the roll cooking of grain sorghum and corn grits, corn is gelatinized to a greater degree than sorghum under the same operating conditions and is more water soluble. Corn grit products exhibit slightly higher amylograph curves than do sorghum grits. If each is processed to the same degree of gelatinization, as measured by a water absorption test, amylograph curves for corn become considerably higher. Low-fat, low-ash flour can be produced by the conventional roller milling of whole grain sorghum. Experimental milling of grain tempered to 19 percent to 19.5 percent moisture produced a 50 to 53 percent extraction of sorghum flour from the first pass. This flour contained 1 percent or less fat and less than 0.5 percent ash. By remilling the shorts, the flour level approached 70 percent extraction without exceeding these fat and ash values.

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Industrial Products

- A. Chemical Composition, Physical Properties and Structure
- B. Chemical and Physical Investigations to Improve Products
- C. Microbiology and Toxicology
- D. Technology--Process and Product Development

Research in these categories is integrated with similar investigations on corn. Results are reported under "Corn Utilization," RPA 407.

RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS
AND NATURALLY OCCURRING TOXINS

B. Microbiology and Toxicology

Research on aflatoxins and other microbiological and toxicological studies relevant to grain sorghum utilization are integrated with similar studies on corn. Results are reported under "Corn Utilization," RPA 702.

RPA 901 - ALLEVIATE SOIL, WATER AND AIR POLLUTION

B. Chemical and Physical Investigations to Improve Products

Research in this category on grain sorghum is integrated with related studies on corn. Results are reported under "Corn Utilization," RPA 901.

Publications - USDA and Cooperative Programs

RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

Chemical Composition and Physical Properties

Waggle, D. H., Deyoe, C. W., and Smith, F. W. (Kansas State University, Manhattan, Kansas). 1967. Effect of nitrogen fertilization on the amino acid composition and distribution in sorghum grain. Crop Sci. 7(4), pp. 367-368.

SOYBEAN UTILIZATION

USDA and Cooperative Programs

Location and Nature of Intramural Work	:Scientist Man-Years FY 1968:				
	: Research Problem Area				Total
	: 406	: 407	: 601	: 901	:
Peoria, Illinois	:	:	:	:	:
Food	:	:	:	:	:
A. Chemical Composition and Physical Properties	: 8.9	:	:	:	: 8.9
B. Flavor	: 10.4	:	:	:	: 10.4
C. Color, Texture and Other Quality Factors	: 2.9	:	:	:	: 2.9
D. Microbiology and Toxicology	: 0.0	:	:	:	: 0.0
E. Technology--Process and Product Development	: 4.0	:	: 2.7	: 0.8	: 7.5
Industrial Products	:	:	:	:	:
A. Chemical and Physical Investi- gations to Improve Products	:	: 6.6	:	:	: 6.6
B. Microbiology and Fermentation	:	: 1.6	:	:	: 1.6
Total	: 26.2	: 8.2	: 2.7	: 0.8	: 37.9

Intramural program is supplemented by extramural support representing (a) 0.0 SMY's at State Agricultural Experiment Stations, (b) 2.9 SMY's at other U.S. institutions^{1/}, and (c) P.L. 480 funds in 4 countries representing 519,159 U.S. dollars equivalent.

- ^{1/} RPA 406: 2.1 (Chemical Composition and Physical Properties, 1.2; Flavor, 0.6; Color, Texture and Other Quality Factors, 0.3)
RPA 407: 0.8 (Chemical and Physical Investigations to Improve Products)

Problems and Objectives

The worldwide need for dietary protein and for food fats poses a problem that urgently demands solution. U. S. soybeans could play a dominant role in alleviating these shortages in developing countries and elsewhere around the world, if soybean protein products can be successfully used in food products tailored to meet the various nutritional and palatability requirements and if opportunities for foreign utilization of soybean oil can be increased by development of inexpensive processes for insuring flavor stability. Solution of these problems would also contribute to increased domestic use of soybean food products and soybean oil. Nonfood usage of soybean oil has rather consistently accounted for about 10 percent of domestic disappearance. In view of the increasing production of soybeans and soybean oil, maintenance of nonfood markets requires continuing effort to develop products that can compete with synthetics derived from nonagricultural sources.

Major objectives of current research are to develop and evaluate alternate ways for:

1. Producing high-protein foods based on full-fat soybean flour.
2. Removing objectionable flavors and other factors from soy products.
3. Increasing flavor stability of soybean oil.
4. Improving refining processes for soybean oil by reducing cost and associated water pollution.
5. Converting soybean oil to competitive industrial chemical products.

Progress - USDA and Cooperative Programs

RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

A. Chemical Composition and Physical Properties

1. Mass spectrometry of glyceride oils and derivatives. Soybean oil volatiles collected over a period of 15 weeks were analyzed by gas chromatography and tandem mass spectrometry. Of 45 components observed in one sample, ethane, pentane, pentene, hexane, diethyl ether, hexene, acetaldehyde, propanol, octane, and octene were among the 22 products which were identified. The mass spectrometer has been successfully used to determine hydrogen evolution during the thermal decomposition of several fatty acid ozonides and to identify the di- and tribasic acids produced.

2. Basic studies on soybean protein. Heating solutions of the 11S protein, major component of isolated soybean protein, at 100° C. and pH 7.6, partly polymerized and partly degraded the protein. Heating for 2-3 minutes at 0.5

ionic strength resulted in the formation of a fast sedimenting aggregate and a slow sedimenting (3-4S) fraction. On continued heating, the aggregate precipitated. In the presence of 0.01 M or 0.5 M mercaptoethanol, the precipitation reaction occurred more rapidly. Lowering the ionic strength in the presence of 0.01 M mercaptoethanol increased the rate of disappearance of the 11S component. At ionic strength 0.5, 11S protein in the presence of 0.01 M N-ethyl maleimide formed a 3S fraction and a soluble aggregate of about 60S which did not precipitate and was quite uniform in size. Viscosity studies indicate that protein conformation is of major importance in influencing electrophoretic mobility of trypsin inhibitor in polyacrylamide gels.

3. Lipoxidase studies. Methods have been devised for the gas-chromatographic analysis of methyl esters dually labeled with C^{14} and tritium which utilize the computer to calculate absolute disintegration rates for the two isotopes. 1- C^{14} -Linoleic acid was incubated with lipoxidase in tritiated water. The product was washed with ordinary water to remove any tritium bound to oxygen and then analyzed to determine the extent of any further incorporation of tritium. The observed ratio of tritium to carbon-14 in the washed product was insignificant.

4. Flatulence factor of soybeans. At the University of Illinois, Urbana, Illinois, under grant research, two dogs with special fistulas (Maydl loops) reacted favorably toward experimentation without the need for anesthetics. It is possible in feeding experiments on these dogs to simultaneously perform mucosal biopsies, bacterial counting, and analyses of the intestinal contents. Soybean meal caused the number and type of microflora in the ileal mucosa to change markedly. Clostridia and gram-positive cocci bacteria increased greatly. These bacteria also produced the greatest amount of gas when incubated with soy flour. Phenolic acid constituents which were previously reported to inhibit flatulence both "in vitro" and "in vivo" do so by temporarily inhibiting growth of gas-producing bacteria.

5. Antinutritional factors in soybeans. Fifty-six different varieties of soybeans were analyzed for fat, moisture, and protein. Complete (except for tryptophan) amino acid analyses of these samples have been initiated. The presence of trypsin inhibitor and hemagglutinating activities is also being studied. This research is being conducted under a grant to the University of Minnesota, Minneapolis, Minnesota.

6. Minor constituents of soybeans. These studies are being conducted under PL 480 grants at foreign institutions. At Kagawa University, Takamatsu, Japan, strains of Escherichia coli have been found which produce significant amounts of alpha-galactosidase activity, the enzyme required for the degradation of raffinose, stachyose, and verbascose, the galactose-containing oligosaccharides of soybeans. The enzyme activity is primarily intracellular. Isolation and purification of the alpha-galactosidase has been accomplished by sonic treatment of the cells to release the enzyme, removal of inert protein by adsorption on calcium phosphate and precipitation of the enzyme

with sodium sulfate followed by adsorption of activity on calcium phosphate and elution of the enzyme.

In research at the Weizmann Institute of Science, Rehovot, Israel, a red protein fraction observed during purification of soybean hemagglutinin has been isolated and identified as cytochrome c. This is only the second cytochrome c isolated from a plant source. The previous plant cytochrome c was obtained from wheat germ. Soybean cytochrome c is found in soybean whey and was purified by chromatography on a cation exchange resin followed by final purification through calcium phosphate column chromatography. Studies on the structure of the glycopeptide isolated from soybean hemagglutinin are being continued.

At the University of Tokyo, Tokyo, Japan, it was found that florisil column chromatography separates soybean sterols into free, glucoside, acylated glucoside and esterified forms of the sterols. The steryl ester and the free sterol fractions, however, contain triglycerides and other materials which interfere with the colorimetric determination of the sterols by a ferric chloride-perchloric acid procedure. Interfering materials are removed by saponifying the sterol esters and precipitating the free sterols with tomatin. The tomatin-sterol complexes are dissociated with dimethyl sulfoxide and subjected to colorimetric determination. This procedure is being applied to the analysis of a number of U.S. and Japanese varieties of soybeans.

7. Triglyceride syntheses. Under a PL 480 grant to the University of Bombay, Bombay, India, purified fatty acids and esters are being prepared for use in the synthesis of di- and triglycerides. Methods of converting purified acids and esters to acid chlorides and mixed anhydrides are being tried to determine the best available route to the pure glycerides.

B. Flavor

1. Selective hydrogenation--homogeneous catalysis. Chromium and molybdenum carbonyl complexes of aromatic and cyclic triene compounds were found to be highly stereoselective for the hydrogenation of conjugated diene and triene fatty esters. The stereoselective 1,4 reduction of trans,trans dienes to cis monoenes was proved by deuterium tracer studies. Soybean oil and esters were effectively hydrogenated with Cr- and Mo(CO)₃ complexes to monoenes with little increase in saturates. Studies with model olefin-iron carbonyl complexes have provided further information on the mechanism of homogeneous catalytic hydrogenation.

2. Selective hydrogenation--heterogeneous catalysis. The activities and selectivity ratios of copper catalysts were found to vary with the type of support employed. The highest activity and selectivity were achieved with copper precipitated on CAB-O-SIL, a pure form of silica with large surface area. Copper-bearing catalysts hydrogenate linolenate 12 to 15 times faster

than linoleate but, unlike nickel catalysts, produce very little saturates. Copper chromite also hydrogenates only those compounds with conjugated systems or with double bonds which are conjugatable. Conjugated trienoates hydrogenate about 50 times faster than conjugated dienoates. Methyl stearolate has been prepared in 80-percent yield and 99+ percent purity by a laboratory procedure which utilizes easily available materials. Hydrogenation of simple binary mixtures with hydrazine has shown that cis-9-, cis-12-, and cis-6-octadecenoic acids reduce at the same rate, but that cis-15-octadecenoic reduces 1.3 times faster than the cis-4 isomer. Cis-3-octadecene was found to reduce 1.3 times faster than cis-9-octadecene.

3. Evaluation of edible soybean oil products. Pentane, released during the thermal decomposition of 13-hydroperoxyoctadeca-9-11-dienoic acid, which is an autoxidation product of linoleic acid in edible oils, shows an inverse linear relationship to flavor and a direct linear relationship to peroxide values. Enzymatic oxidative polymers formed along with the peroxide also yield pentane as the only hydrocarbon. Long-term storage for 6 months or more at ambient and elevated temperatures has shown that hydrogenated winterized soybean oils, protected by nitrogen, remains free of peroxides, retains high flavor scores, and shows little if any loss of quality. Trace amounts of high-molecular-weight hydrocarbons and insecticidal contaminants of soybean oil can usually be removed in laboratory simulations of commercial processing procedures.

4. Flavor components of soybean meal and protein. Research at the University of Tokyo, Tokyo, Japan, under a PL 480 grant, indicated that a crude enzyme preparation from aspergillopeptidase A partially digests soybean protein curd and defatted soybean flour. Degree of hydrolysis as measured by the ratio of trichloroacetic acid-soluble nitrogen to total nitrogen was about 0.5. Free amino acids were formed in very small amounts and could be completely removed by washing with 90 percent ethanol. Washed product contained no beany flavor. It was considered to be superior in flavor to starting material and suitable for foods.

C. Color, Texture and Other Quality Factors

1. Minor constituents of soybeans. Studies of lipid degradation in soybean meal during meal preparation showed that very little degradation occurred and that undesirable flavor characteristics of soy flour may not be derived from this source. Defatted flakes extracted with a hexane-ethanol azeotrope had very little flavor and had a nitrogen solubility index of 80. The extracted lipids had very pronounced flavors and were highly susceptible to oxidative degradation. The procedures used in preparing soy protein concentrates and isolates from defatted meal may accelerate lipid oxidation.

2. Color reversion of edible soybean oil. At the Sugiyama Chemical Research Institute, Tokyo, Japan, the tocopherol content of extracted soybean oil was correlated with moisture content of the beans, and the distribution of tocopherol in the cotyledon cells was studied. Tocopherol was

also found in cellular particles and cytoplasmic solution that did not contain depot lipids. The tocopherol content of cotyledons did not decrease when the soybeans absorbed a large amount of water by imbibition or germination. No change was observed in the concentration of α tocopherol but both γ and δ showed a marked decrease. It was postulated that moisture caused conversion of tocopherol into dimers in the absence of oxidizing enzymes. A dimeric tocopherol was in fact isolated from soybean oil and identified. These results suggest that color reversion in soybean oil can be explained on the basis that the dimeric tocopherol, a highly labile compound is extracted with the oil and rearranges to tocopherol upon aging of the oil. These studies are being conducted under a PL 480 grant.

D. Microbiology and Toxicology

1. U.S. soybeans for making tofu. Studies on the use of U.S. soybeans in making tofu are being conducted at several foreign institutions under PL 480 grants. At the University of Tokyo, Tokyo, Japan, laboratory procedures for making dried tofu (kori-tofu) have been found that simulate commercial procedures. Speed of stirring during coagulation affected particle size of curd; the greater the speed, the smaller the size. Cause of browning of kori-tofu was shown to be related to oxidation of fat that occurs during storage at high relative humidities and temperatures. Carbonyl compounds are formed that accelerate browning. Storage at -27 and $+5^{\circ}$ C. effectively preserved the excellent properties of ammonia-treated kori-tofu, but storage at 25 and 45° C. did not. Twenty-two samples of U.S. soybeans were tested for swelling quality and coagulation in preliminary work on making kori-tofu from them.

Experiments were made at the Tokyo University of Education, Tokyo, Japan, to produce a cheese-like food with good flavor, texture, and superior keeping quality from soy milk. One step in the process involves aging with a proteolytic enzyme. Of the commercial enzymes tried (trypsin, molsin, papain, bioprase, and pronase), papain, because of its price and activity at pH 6, appeared to offer the most promise. The best cheese-like products were made from soybean curd to which some dairy cheese was added to produce a product resembling processed cheese.

Concurrent studies were made on soybean proteins to separate the trypsin inhibitors. Water-extractable soybean proteins were separated by gel filtration with Sephadex G-200 into four fractions. The second fraction was ultracentrifugally homogenous, showed trypsin inhibition activity, and differed from the three other fractions in amino acid composition, especially in the higher content of sulfur-containing amino acids. By means of DEAE-cellulose column chromatography with gradient elution, the second fraction was fractionated into six fractions that consisted of proteins having different ratios of trypsin inhibition to chymotrypsin inhibition.

In research completed during the year by the Japan Tofu Association, Tokyo, Japan, the interactions between soybean protein, calcium and phytate were

studied by gel filtration and solubility measurements to gain basic information about reactions occurring during tofu-making. Calcium, phytate and protein were not separated from each other when calcium precipitated protein was analyzed by gel filtration. Evidently a strong interaction occurs between these components. Measurement of solubility as a function of pH confirmed that the three components react with each other. In the absence of phytate, calcium did not appear to react with protein. Addition of phytic acid during preparation of tofu increased water-holding capacity and yield and decreased hardness of tofu as compared to a control with no added phytic acid. Several soybean varieties are being studied to determine whether there is a relationship between phytic acid content and suitability for tofu-making.

2. Studies on miso and shoyu. As a result of research conducted under a PL 480 grant at the Noda Institute for Scientific Research, Noda-shi, Chiba-ken, Japan, diploid Saccharomyces rouxii cultures can now be successfully produced in almost every combination with opposite mating types (haploids). The diploids with characteristics from each parent may be kept in the diploid condition indefinitely. Some diploid strains show better aerobic growth than do the parent haploids and also produce higher yields of polyalcohol. No improvement of the diploids over the haploids has yet been accomplished in respect to characteristics such as salt tolerance, ethanol production, and fermentation rate. The new diploids are stable when grown in shoyu mashes containing 18 percent sodium chloride. Therefore, when desirable diploids are made, they will retain this condition indefinitely in the shoyu mashes without sporulation or reversion to their parental types. Diploids can be made to sporulate on a 5-percent diluted shoyu-koji medium if 5 percent of glucose is added.

Trials in testing mills, conducted under a PL 480 grant by the Japan Shoyu Research Institute, Tokyo, Japan, showed no apparent difference between Japanese and American soybeans for making shoyu. Both fermented without requiring more care than other raw materials. Problems with fines and clods were encountered with both Japanese and American soybean meals in plant and laboratory tests. More uniform spraying with water and additional cooking appeared to be needed. Alcohol-washed meal gave high yields of total nitrogen in the 3-month-old Moromi juice but the percentage yield (80.6%) of juice was not much higher than that given by other raw materials. Use of more saline water might improve yields. Further trials with grits failed to find methods of using them. In laboratory tests, defatted American soybeans yielded more total nitrogen and glutamic acid in shoyu (soy sauce). Since there was little if any organoleptic difference between shoyus prepared from Japanese and American defatted soybeans, the latter appear to be better suited to shoyu manufacture.

3. Chinese cheese (sufu). The Institute of Chemistry, Academia Sinica, Taipei, Taiwan, has identified Actinomucor elegans as the species used commercially to make Chinese cheese. Other species of Mucor (M. silvaticus,

M. subtilissimus, and M. hiemalis) are used in the home. Improvements in making sufu included the preparation of drier tofu containing 77.6 percent moisture and, to reduce bacterial growth, immersion of tofu in a salt-acid solution prior to inoculation with the mold. Inoculation was carried out with a spore suspension on filter paper. For ideal fermentations, the temperature should be about 20° C. Various satisfactory methods for preserving Chinese cheese, besides canning, were developed, including coating of the cheeses with paraffin and storage under inert gases. Biochemical analysis of sufu showed that soybean protein was partially digested, that more free fatty acids were present, and that water-soluble nitrogen compounds increased during brining after death of the mold. Also present in sufu are enzymes produced by Actinomucor. These are trypsin, chymotrypsin, phospholipases, and pepsin-like enzymes including dipeptidases. The sufu contains more free fatty acids. This research, now completed, was conducted under a PL 480 grant.

E. Technology--Process and Product Development

1. Pilot-scale selective hydrogenation. A continuous method for removing copper from copper-chromite hydrogenated soybean oil has been devised. The method involves passing the oil countercurrent to deionized water and removing cations in the water with a hydrogen-form cation exchange resin. Copper content was reduced to 0.03 p.p.m., a level typical of refined, bleached, unhydrogenated soybean oil. Hydrogenation of soybean oil with a commercial copper-chromium catalyst to a 0.1 percent linolenate level followed by winterization gave a 74-percent yield of oil which passed a 20-hour cold test. When tasted after 4 and 8 days' storage at 60° C., the copper hydrogenated-winterized oil showed significantly better flavor stability than did the unhydrogenated soybean oil. With nickel catalyst, hydrogenation to a 2.3 percent linolenate level gave only a 39-percent yield of winterized oil.

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Industrial Products

A. Chemical and Physical Investigations to Improve Products

1. Aldehyde oil derivatives. Studies on two potential monomers for nylon-9, 9-aminononanoic acid and 9-aminononanamide, indicate the amino acid is preferable. Preparation of the amino acid from pure methyl azelaaldehyde (MAZ) was successfully increased from 0.1 molar to 1.3 molar quantities with a total yield of polymerizable monomers, including amino ester and aminoamide, of over 90 percent. Steps were taken to simplify reaction and processing steps for the synthesis of nylon-9 from soybean oil. MAZ of high purity was isolated in good yield using a poisoned palladium catalyst in place of palladium on charcoal.

Although an apparently satisfactory degree of crosslinking of poly(ester-acetals) and poly(amide-acetals) was obtained under ideal conditions at 100-150° C., practical test conditions required a temperature of at least 200° C. At this temperature, p-toluenesulfonic acid alone gave as good results as any catalyst-promoter combination. Cured coatings on metal of a 50-percent pentaerythritol acetal copolymer showed superior impact properties over commercial polyester and epoxy coatings. High surface hardness was obtained only under conditions which caused discoloration of the copolymer. These studies, conducted under a contract by Fabric Research Laboratories, Dedham, Massachusetts, have been completed.

2. Homogeneous catalytic hydrogenation. Studies under a grant to the University of Illinois, Urbana, Illinois, on hydrogenation of model dienes and monoenes with platinum-phosphine/arsine-tin complexes show the reaction to be dependent upon both the double bond position and the length of the carbon chain. Terminal double bonds and short-chain monoenes are the most easily reduced. Methyl substitution near the double bond inhibited hydrogenation. Some short-chain conjugated dienes may form strong unreactive pi-complexes with the catalyst, resulting in inactivation. Studies with deuterium gas and deuterated methanol showed that the acidic proton of methanol participated in the isomerization of 1,5 hexadiene.

3. Isomerization studies. Under a PL 480 grant to the University of London, London, England, model compounds for use in studies on isomerizations have been prepared. Base catalyzed isomerizations have been carried out and the composition of the products is being analyzed. Trans-hex-3-enoic acid in 7N potassium hydroxide gives 56 percent of the trans-hex-2-enoic acid, 39 percent trans-hex-3-enoic acid, and 5 percent cis-hex-2-enoic acid. Hydrocarbons containing the cis,cis 1,4-pentadiene or related polyene systems such as trideca-5,8-diyne and trideca-cis-5, cis-8-diene, have been prepared for study.

4. Cyclic fatty acids. Research on cyclic fatty acids is relevant to soybean utilization. Results are reported under "Flax Utilization," RPA 407, Subheading B-1.

B. Microbiology and Fermentation

1. Microbial modification of fatty acids. The optical rotation of the methyl ester of the hydroxy fatty acid formed from oleic acid by fermentation with strains B-2994 and B-3266 was determined. The rotational value for the product formed by B-2994 was indicative of a stereospecific enzyme, while that for the product formed by B-3266 indicated a nonspecific activity. Introduction of an oxygen function exclusively at the 10-position by the fermentative modification of oleic acid permits introduction of other reactive groups at this location. For example, the oximes of 10-ketostearic acid and its methyl ester have been prepared. Also, 10-aminostearic acid has been synthesized and polymerized. This polymer appears capable of fiber

formation. Fermentations of linoleic acid and ricinoleic acid with both B-3266 and B-2994 have shown the presence of new products which are being separated for purposes of purification, characterization, and analysis.

2. Lipolytic enzymes. Under a PL 480 grant at the University of Baroda, Baroda, India, potent lipolytic microorganisms have been found in natural sources. Most of the research to date has been done on strains of Pseudomonas aeruginosa. Studies on the nutritional and environmental factors required for maximum lipase production have been completed. The lipase from one strain has been partially purified and its action pattern on fatty substrates has been established. This lipase acts predominantly at the primary alcohol positions of triglycerides and leaves a monoglyceride as a product of the enzymatic reaction. In this respect it is quite similar to the well-known pancreatic lipase of commerce.

RPA 601 - EXPANSION OF FOREIGN MARKETS FOR U.S. PRODUCTS

E. Technology--Process and Product Development

1. Full-fat soybean flour. A statistically designed experiment on the production of full-fat soy flours by extrusion cooking shows significant relationships between processing conditions and nutritional properties, functional properties, flavor, odor, and oxidative stability. Rat-feeding tests showed that the PER value of the flour was 2.2 (casein = 2.5). A progressive increase in PER values was found with respect to trypsin inhibitor deactivation up to 89 percent. When deactivation exceeded 89 percent, PER values decreased. When the flours were used in soy beverages, all residual trypsin inhibitor activity was destroyed by cooking for 20 minutes, the optimum time of cooking for taste panel acceptability.

RPA 901 - ALLEVIATE SOIL, WATER AND AIR POLLUTION

E. Technology--Process and Product Development

1. Refining soybean oil. Work has recently been undertaken to develop a lower cost process for refining soybean oil that will also reduce stream pollution by minimizing refinery wastes. Reportable results have not yet been obtained.

Publications - USDA and Cooperative Programs

RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

Chemical Composition and Physical Properties

Aspinall, G. O., Begbie, R., Hamilton, A., and Whyte, J. N. C. (University of Edinburgh, Edinburgh, Scotland). 1967. Polysaccharides of soy-beans. Part III. Extraction and fractionation of polysaccharides from cotyledon meal. J. Chem. Soc., Sec. C(11), pp. 1065-1070.*

*Research supported by PL 480 funds.

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*Research supported by PL 480 funds.

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Flavor

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Technology--Process and Product Development

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RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Industrial Products

Chemical and Physical Investigations to Improve Products

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RPA 601 - EXPANSION OF FOREIGN MARKETS FOR U.S. PRODUCTS

Technology--Process and Product Development

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*Research supported by PL 480 funds.

FLAX UTILIZATION

USDA and Cooperative Programs

Location and Nature of Intramural Work	:Scientist Man-Years FY 1968:	
	: Research Problem Area	: Total
	407	
Peoria, Illinois		
<u>Industrial Products</u>		
A. Chemical Composition, Physi- cal Properties and Structure	3.3	3.3
B. Chemical and Physical Invest- igations to Improve Products	6.8	6.8
C. Microbiology and Fermentation	0.7	0.7
D. Technology--Process and Product Development	0.4	0.4
Total	11.2	11.2

Intramural program is supplemented by extramural support representing (a) 0.0 SMY's at State Agricultural Experiment Stations, (b) 0.8 SMY's at other U.S. institutions^{1/}, and (c) P.L. 480 funds in 2 countries representing 37,466 U.S. dollars equivalent.

1/ RPA 407: 0.8 (Chemical and Physical Investigations to Improve Products)

Problems and Objectives

Traditional markets for linseed oil, the major drying oil produced and used in the United States, are threatened by widespread use of synthetic products derived from nonagricultural sources. Recently, annual domestic use of linseed oil has declined to 325 million pounds from a postwar high of over 700 million pounds in 1950. This decrease came primarily by displacement by synthetic materials capable of better performance, particularly in coatings. To restore the level of use of linseed oil, new or expanded markets are urgently needed. The most promising route to achievement of this goal is development of improved protective coating products that can compete with synthetics. Other new outlets can be realized by chemical modification of linseed oil to obtain materials that will find applications in the multi-billion-pound annual market for products of the organic chemical industry.

Major objectives of current research are to develop and evaluate alternative ways for:

1. Improving durability of linseed emulsion paints.
2. Establishing economically feasible methods of curing and/or protecting concrete by use of linseed oil.
3. Converting linseed oil by economical processes to products that can compete in the industrial chemical market.

Progress - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Industrial Products

A. Chemical Composition, Physical Properties and Structure

1. Linseed oil films and emulsions. Swelling of unpigmented linseed oil film in buffer solutions increases with increasing pH values above 6. The films eventually decompose above pH 9. ZnO, an alkaline pigment, may be responsible for swelling of films from linseed oil paints. An aqueous slurry of ZnO and TiO₂ (pH 9.1) caused linseed oil films to swell by 18 percent. Treatment of ZnO with inorganic phosphate reduced the pH of the aqueous slurry to 8.5 and swelling of the film pigmented with this material to 13 percent. With organic phosphate treatment of ZnO, pH is 7.6 and swelling is 11 percent. For pH values between 2.2 and 6, no swelling of unpigmented films was observed. Statistically designed experiments showed swelling to be a function of alkalinity and to be unaffected by the presence of either sodium or zinc ions.

The spray-drying technique for preparing oil-coated pigments was not entirely successful since many pigment aggregates, some greater than 10 μ , were formed. A new procedure, involving dispersion of the pigments in a solution of linseed oil and hexane followed by emulsification and hexane evaporation, has enabled preparation of suspensions of oil-coated pigments suitable for formulating linseed emulsion paints that yield glossy films. Tests have shown that experimental cationic linseed oil emulsion paints achieved dry-to-touch characteristics in 15 to 20 minutes, water resistance in 20 to 30 minutes, and tack-free finish in 1 to 3 hours. Cationic paints passed a 24-hour water-soaking test after 24 hours air drying, but all commercial water-based paints tested failed the same test.

B. Chemical and Physical Investigations to Improve Products

1. Cyclic fatty acids. Simple thermal decomposition in vacuo of the ozonized ethylene adduct of methyl linoleate produces an ester acid in good yield (76%) and with subsequent hydrolysis provides a convenient route to the corresponding tribasic acid. A blend of the cyclohexyl ester of HCal (hydrogenated cyclized linseed fatty acids) and the 2,2,4,4-tetramethyl-1,3-cyclobutanediol diester of HCal was tested and met all viscosity requirements of the military specifications for jet lubricants. However, sludge formation was above the maximum allowed. It should be possible to eliminate this difficulty by use of inhibitors designed for use with HCal esters.

2. New polymers and derivatives for coatings. Polyesteramides containing 10, 20, and 30 mole percent excess of N,N-bis(2-hydroxyethyl) linseed amide (HELA) over isophthalic acid were modified with the stoichiometric amounts of toluene diisocyanate (TDI). In systems containing drier, increasing the amount of TDI shortened the drying times in air and increased alkali resistance, but did not significantly improve hardness. Films were examined for yellowing in both darkness and north light. Initially, films from polyesteramides modified with hexane 1,6-diisocyanate (HDI) or dicyclohexylmethane 4,4'-diisocyanate (HMDI) were less yellow than those modified with TDI, but at 3 months HDI and HMDI films were equal in color to TDI films. Films yellowed slightly more in the dark than in north light. Hydrogen-sulfide-treated linseed oil containing 8.8 percent sulfur was reacted with an equal amount of TDI in DMSO at 110° for 2 hours. Preliminary results on films baked for 30 minutes at 200° C. showed 7 hours alkali resistance compared to 1/2 hour for the linseed oil control.

Studies on new linseed oil derivatives for protective coatings are being conducted under a cooperative agreement among the Northern Division, the National Flaxseed Processors Association, and North Dakota State University, Fargo, North Dakota. Mercurated linseed oil and linseed methyl esters released free mercury during 6-month storage tests. Improved stability is being sought. Copolymers of hydroxy ethyl acrylate, other acrylate esters, and styrene gave products with pendant hydroxyl groups which reacted with acetal oils (from ozonization of linseed oil) to form excellent films.

Sward hardness was 18 to 26 after 12 hours and 54 to 56 after 7 days of drying. Linseed oil preheated to 320° C. and passed through a eutectic mixture of 54.5 percent KNO₃ and 45.5 percent NaNO₃ at 280 to 320° C. gave oils having a viscosity of 0.85 to 4.70 stokes and decreased its solubility in hydrocarbon to 70 percent. Attempts to add iodo- and chloroisocyanates to oleic acid and methyl oleate resulted in compounds showing both chemical reactivity and infrared absorption spectra characteristic of isocyanates.

3. Catalytic conjugation. Studies on the conjugation of methyl linoleate and linolenate using palladous chloride, rhodium trichloride, and dichlorobis (triphenylphosphine) platinum-stannous chloride (DPC) indicate that rhodium trichloride and DPC retain stability at the required temperature of 150° C. necessary for reasonable reaction rates. When methyl linoleate was heated with DPC for 24 hours at 150° C. in *n*-butyl carbitol solution, distillation yielded volatile esters containing about 60 percent of conjugated diene. Volatile esters from methyl linolenate isomerized under the same conditions contained about 48 percent of conjugated triene. When DPC was used with linseed or soybean oils without solvent, about 27 percent and 20 percent of conjugation, respectively, were produced.

4. Photochemistry of linseed oil polymers. In grant research at the University of Illinois, Chicago, Illinois, the problem of thermal oxidation of linseed oil on metal oxide substrates was examined by differential thermal and thermogravimetric analyses. Linseed oil showed a strong exothermic reaction on all substrates and in the bulk oil at 150° C. The reaction is an oxidation of an unsaturated moiety by atmospheric oxygen rather than by surface oxide. These results indicate that photo-induced oxidation can be separated from thermal reactivity at temperatures below 150° C.

5. Hydroxylation of linseed oil. At the Regional Research Laboratory, Hyderabad, India, evidence was obtained showing that sultone formation takes place during sulfation of linoleate. The precursor appears to be trans,trans conjugated isomers formed from linoleate under the influence of strong mineral acid. Cis,trans isomers, also present, undergo normal sulfation. Autoxidation of linseed oil followed by reduction of hydroperoxide groups to hydroxyl groups yielded a product having unaltered iodine value and a hydroxyl value of 50. Bromination of safflower oil in the presence of ultraviolet light gave a product in which all bromine could be replaced by reaction with silver oxide. Conversion to hydroxyl was low owing to formation of ethers. Products having hydroxyl values of 168 and 105, respectively, were prepared by oxidation of safflower and linseed oils with selenium dioxide. This research is being conducted under a PL 480 grant.

C. Microbiology and Fermentation

Research in this category is integrated with similar investigations on soybeans. Results are reported under "Soybean Utilization," RPA 407, B.

D. Technology--Process and Product Development

1. Linseed oil coatings for concrete. Final results on contract studies at Kansas State University, Manhattan, Kansas, showed that concrete beams cured with the boiled linseed oil emulsion developed at the Northern Division, when subjected to freeze-thaw tests in 2 percent brine, were less damaged after 300 cycles than air-cured beams. Superiority of the oil-cured beams disappeared after 403 cycles. Based on these tests, the optimum coating rate is 1 gallon of emulsion per 225 sq. ft., and optimum application time is 4 to 6 hours after placing the concrete. Both the torque wrench test and the California abrasion test demonstrated that specimens cured with boiled linseed oil emulsion and aged at 70° F. and 50-percent relative humidity had surface strengths superior to those of sheet-cured specimens, which in turn were superior to air-cured specimens. No differences in freeze-thaw durabilities were found among the samples when aged for 6 weeks at 70° F. and 50-percent relative humidity.

Publications - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Industrial Products

Chemical Composition, Physical Properties and Structure

Fedeli, E., Capella, P., Cirimele, M., and Jacini, G. (Experiment Station for the Fats and Oils Industry, Milan, Italy). 1966. Isolation of geranyl geraniol from the unsaponifiable fraction of linseed oil. J. Lipid Res. 7(3), pp. 437-441.*

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*Research supported by PL 480 funds.

Chemical and Physical Investigations to Improve Products

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- Schwab, A. W., Gast, L. E., and Cowan, J. C. 1968. Free radical addition of hydrogen sulfide and thiols to linseed oil and methyl oleate. J. Amer. Oil Chem. Soc. 45(6), pp. 461-464.

Technology--Process and Product Development

- Faulkner, R. N., and Berry, E. E. (The Research Association of British Paint, Colour, and Varnish Manufacturers, Teddington, Middlesex, England). Sept. 26, 1967. Compositions for increasing the dispersion stability of titanium dioxide pigment. U.S. Patent 3,343,974.*
- Faulkner, R. N., and Sen, N. (The Research Association of British Paint, Colour, and Varnish Manufacturers, Teddington, Middlesex, England). Nov. 21, 1967. Pigment dispersants comprising phosphonic acid diols-alder adducts of vegetable oil materials. U.S. Patent 3,354,104.*

*Research supported by PL 480 funds.

Kubie, W. L., Gast, L. E., and Cowan, J. C. 1968. Preliminary report on skid resistance of linseed oil-coated concrete. In "Surface Properties of Pavements and Vehicle Interaction," Highway Res. Rec. No. 214, pp. 42-49. Washington, D. C.

CRAMBE UTILIZATION

USDA and Cooperative Programs

Location and Nature of Intramural Work	:Scientist Man-Years FY 1968:	
	: Research Problem Area	:Total
	407	
Peoria, Illinois		
<u>Feeds</u>		
A. Chemical Composition and Physical Properties	5.4	5.4
B. Technology--Process and Product Development	1.8	1.8
<u>Industrial Products</u>		
A. Chemical and Physical Investigations to Improve Products	3.4	3.4
Total	10.6	10.6

Intramural program is supplemented by extramural support representing (a) 0.0 SMY's at State Agricultural Experiments, and (b) 1.1 SMY's at other U.S. institutions^{1/}.

^{1/} RPA 407: 1.1 (Industrial Products: Chemical and Physical Investigations to Improve Products, 0.5; Technology--Process and Product Development, 0.6)

Problems and Objectives

Crambe, a new oilseed crop commercialized in 1965, is the first plant included in the research program on new crops to achieve this status. Crambe seed oil is rich in erucic acid. Several industrial uses already exist for erucic acid as well as for imported rapeseed oil, which formerly was the only source of this acid. However, to insure optimum development of crambe as a new commercial crop, possible markets for crambe oil and erucic acid must be explored and those with the greatest industrial potential must be identified and exploited. In addition, since economic value to the farmer and to industry of any oilseed crop is much greater if the meal left after extraction of the oil can be utilized as a palatable and nutritious feed for animals, suitable processes are required to realize fully the anticipated nutritional qualities and to insure maximum acceptability to different types of animals.

Major objectives of current research are to develop and evaluate alternate ways for:

1. Developing new plastics, resins, and special-purpose plasticizers from crambe oil.
2. Improving protein feed supplements from crambe.

Progress - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Feed

A. Chemical Composition and Physical Properties

1. Crambe enzymes. Seed meals from various species of Cruciferae were surveyed for the production of nitriles and thionamides by the action of ferrous salts. Results showed that a hydroxyl group in the 2-position of an alkyl glucosinolate is essential for thionamide formation. Thioglucosides lacking such a hydroxyl group produced only nitriles. Iron in three crambe meals increased 15 to 100 p.p.m. when the meal was ground in an iron hammer mill. As expected, little change in the thioglucoside hydrolysis products occurred since little of the introduced iron is likely to be present as ferrous ions. The thioglucosidase from crambe was solubilized by sonication and chromatographed on a G-100 Sephadex column. Results indicate a molecular weight greater than 70,000.

2. Conversion products from epi-progoitrin (epi-PG). Rat feeding experiments with crambe meals showed that both meal containing epi-progoitrin (epi-PG) and active enzymes and meal autolyzed to form nitriles were highly

toxic. Meals containing either epi-PG and inactivated enzymes or (R)-goitrin were less toxic. Tests on mice with each of three isolated nitriles showed that LD₅₀ ranged from 145 to 237 mg./kilo of body weight. The LD₅₀ for (R)-goitrin exceeded 1,260 mg./kilo. A gas chromatographic method for more rapid and specific determination of nitriles, goitrin, and thionamides has been developed and is being tested. Two nitro olefins have been synthesized from glucose. Either of these can be used in synthesis of epi-progoitrin.

B. Technology--Process and Product Development

1. Processing crambe to oil and meal. Crambe meal samples treated by a procedure involving both a cook with soda ash and extraction with water gave excellent results in a rat feeding trial but performed less well in chick feeding studies. Further investigation is necessary to determine the cause of the poorer response in chicks. New chick feeding trials that should eliminate nutritional factors and permit evaluation only of toxic factors in crambe meals have been designed and initiated.

In trials conducted under a cooperative agreement by the University of Nebraska, North Platte, Nebraska, crambe meals treated with moist heat, with or without soda ash, gave good results as feed supplements. There were no apparent ill effects on the animals. Daily gains were 92-93 percent of values obtained with soybean meal. A slight unpalatability factor still remains in the crambe meal, but this factor should not be significant if the crambe meal is blended with other meals in preparing the supplement.

Industrial Products

A. Chemical and Physical Investigations to Improve Products

1. Chemical modification of crambe oil. Twenty-nine esters of brassylic acid and esters of mixed dibasic acids derived from crambe fatty acids have received preliminary evaluation and, on the basis of viscosity properties and a standard anti-wear test, several appear to have potential as hydraulic fluids, aircraft engine lubricants, and helicopter transmission fluids. A 96-percent recovery of 98-percent pure brassylic acid was obtained from a synthetic mixture of brassylic and pelargonic acids using Freon 113 as a solvent. Norit EX, a decolorizing charcoal, removed color and color-forming trace impurities from brassylic acid in ethanol solution. On the basis of inspection before and after heating at 205° C. for 2 hours, the purified product was comparable in color and color stability to commercial polymer-grade azelaic acid.

2. Polyamide resins from erucic acid. In contract research at Southern Research Institute, Birmingham, Alabama, a new synthetic route to nylon-13 which provides an overall yield of over 50 percent based on erucic acid amide was developed and used to synthesize 500 g. of polymer. Properties of nylon-13 are generally similar to those of nylon 1313 but seem less

influenced by molecular weight. Nylon-13 has slightly higher hardness and tangent modulus of elasticity but slightly lower tensile and flexural strengths than similar specimens of nylon 1313. Nylon 613 was prepared from brassylic acid and 1,6-diaminohexane. It had good physical properties and resembled nylon 610 as a molding resin.

Publications - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Feed

Chemical Composition and Physical Properties

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Kwolek, W. F.,¹ and VanEtten, C. H. (¹USDA Biometrical Service, Peoria, Illinois). 1968. Evaluation of amounts and pattern of essential amino acids in plant seeds. J. Agr. Food Chem. 16(3), pp. 495-499.

VanEtten, C. H., Kwolek, W. F.,¹ Peters, J. E., and Barclay, A. S.² (¹USDA Biometrical Service, Peoria, Illinois; ²USDA Crops Research Division, Beltsville, Maryland). 1967. Plant seeds as protein sources for food or feed. Evaluation based on amino acid composition of 379 species. J. Agr. Food Chem. 15(6), pp. 1077-1089.

Wolff, I. A. 1967. Crambe vegetable oil. In "Encyclopedia of Basic Materials for Plastics," ed. Herbert R. Simonds and James M. Church, Reinhold Publishing Corp., New York, pp. 129-130.

Technology--Process and Product Development

Mustakas, G. C., Kirk, L. D., Griffin, E. L., Jr., and Clanton, D. C.¹
(¹University of Nebraska, North Platte, Nebraska). 1968. Crambe seed processing. Improved feed meal by soda ash treatment. J. Amer. Oil Chem. Soc. 45(1), pp. 53-57.

Industrial Products

Chemical Composition and Physical Properties

Miwa, T. K., Chang, S. P., Tallent, W. H., Wolff, I. A., Palm, W. E.,¹
and Witnauer, L. P.¹ (¹Eastern Utilization Research and Development
Division, Philadelphia, Pennsylvania). 1967. Internally plasticized
copolymers of vinyl chloride and 2-methylpentyl vinyl brassylate.
ACS Polymer Preprints 8(2), pp. 927-933.

Chemical and Physical Investigations to Improve Products

Chang, S. P., Miwa, T. K., and Wolff, I. A. 1967. Alkyl vinyl esters of
brassylic (tridecanedioic) acid. J. Polym. Sci., Part A-1, 5(10),
pp. 2547-2556.

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Preparation and evaluation of surface-active brassylic acid-ethylene
oxide adducts. J. Amer. Oil Chem. Soc. 45(3), pp. 159-164.

Nieschlag, H. J., Tallent, W. H., Wolff, I. A., Palm, W. E.,¹ and
Witnauer, L. P.¹ (¹Eastern Utilization Research and Development Division,
Philadelphia, Pennsylvania). 1967. Diester plasticizers from mixed
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NEW CROPS UTILIZATION

USDA and Cooperative Programs

Location and Nature of Intramural Work	:Scientist Man-Years FY 1968:		
	: Research Problem Area		: Total
	: 407	: 702	:
Peoria, Illinois	:	:	:
<u>Feed</u>	:	:	:
A. Chemical Composition and Physical Properties	: 1.1	:	: 1.1
<u>Industrial Products</u>	:	:	:
A. Chemical Composition, Physi- cal Properties and Structure	: 8.1	:	: 8.1
B. Microbiology and Fermentation	:	: 1.4	: 1.4
C. Technology--Process and Product Development	: 1.2	:	: 1.2
Total	: 10.4	: 1.4	: 11.8

Intramural program is supplemented by extramural support representing P.L. 480 funds in 1 country representing 66,344 U.S. dollars equivalent.

Problems and Objectives

Farmers could achieve more economic use of their land if new and profitable crops were available for their choice that would have different end-use patterns from those presently grown. To develop a new crop, three basic steps are involved: (1) survey of wild plants to identify those having both potentially valuable components and promising agronomic potential for use in the United States; (2) detailed physical and chemical studies on components of interest to obtain clues to likely end uses; and (3) selection of the most promising species, followed by additional utilization research to explore uses and demonstrate industrial potential. Close cooperation is needed with plant scientists who provide assistance and advice in acquisition and selection of samples for screening and who have responsibility for subsequent agronomic research such as establishing proper cultural practices and identifying the best strains and varieties.

Major objectives of current research are to develop and evaluate alternate ways to:

1. Identify new plant sources for industrial vegetable oils from among samples collected worldwide.
2. Determine utilization potential of oils and feed meals from selected new oilseeds.
3. Produce marketable rotenoid products from the domestic legume Tephrosia vogelii.
4. Produce papermaking pulp from kenaf.

Progress - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Feed

A. Chemical Composition and Physical Properties

Research in this category is integrated with similar investigations on crambe. Results are reported under "Crambe Utilization," RPA 407, Feed, subheading A.

Industrial Products

A. Chemical Composition, Physical Properties and Structure

1. Screening for new industrial oils. The seed collection was increased by 792 accessions and the number of identified species by 283. Analyses were made on 638 seed samples and 348 seed oils, in addition to 423 Vernonia samples analyzed in support of a Crops Research Division contract at a State Agricultural Experiment Station. Unusual oils found include one with 73 percent hydroxy acid (not ricinoleic), one with 62 percent C_{18} acids, and two with 21 and 24 percent cis-5-octadecenoic acid. A previously reported unknown acid was identified as dihydrosterculic acid (17%). A number of oils were found by GLC to contain 20-90 percent of materials more volatile than the usual triglycerides. One contained 35 percent of myristicin (5-methoxysafrole). Vernolic acid was found in 37 new accessions of Vernonia, other than V. anthelmintica. Crepenynic acid (18-49% of the seed oil acids) was found in one new genus and two new species of previous genera. The highest proportion of petroselinic acid found in oils of 146 Umbelliferae species was 86 percent; oil from 8 species contained none. Ten of 65 Brassica samples contained oil with at least 55 percent of erucic acid. An "interrupted ozonolysis" procedure was devised to facilitate identification of unsaturated acids, including polyenes with double bonds in unusual locations.

In studies completed during the year under a PL 480 grant at the Swedish Seed Association, Svalof, Sweden, analysis of oils from 75 self-pollinated plants of Brassica carinata showed that erucic acid content ranged from 27 to 43 percent. Seed from plants highest in erucic acid content were planted for further selection. It is believed the erucic acid contents for crop year 1966 are low because of unusual weather conditions. The range of erucic acid in B. napus oil (44 lines) was from 35 to 52 percent. Treatment of B. hirta with a mutagenic agent failed to develop plants with more than 67 mole percent erucic acid in the oil. Six plants out of the 24 had 55 percent erucic or more. B. campestris, eleven varieties from India, and B. carinata, 25 individual plants, produced the same range of isothiocyanates, 11.3 to 17.4 and 9.3 to 17.9 mg./g. dry meal, respectively. B. napus, 81 strains, produced 1.8 to 14.2 mg./g. of isothiocyanates and 8.4 to 22.8 mg./g. of oxazolidinethione (3.9 to 9.6% total thioglucosides in the dry, solvent-extracted meal).

Analyses of 1967 crop samples are not yet available.

2. Characterization of seed oils and component fatty acids. A previously unknown hydroxy fatty acid found in the seed oil of Thymus vulgaris L. in 13-percent yield has been structurally characterized as (-)- α -hydroxylinolenic acid. Trihydroxy acids with two of the hydroxyl groups acetylated are found attached exclusively to the β -position of the glycerol moiety in Chamaepeuce afra glycerides. Four oxygenated fatty acids, isolated from oil

of several foreign introductions of sunflower seed have been characterized as cis-9,10-epoxystearic (0.5%), cis-9,10-epoxy-cis-12 octadecenoic (2.2%), 13-hydroxy-cis-9,trans-11-octadecadienoic (1.3%), and 9-hydroxy-trans-10,cis-12-octadecadienoic (1.2%) acids. Autoxidation of oils from four Russian varieties of sunflower seeds under prevailing laboratory conditions is slower than autoxidation of oils from other sunflower seed oils examined.

3. Rotenoid determinations in Tephrosia vogelii. A solvent system for countercurrent distribution has been found that effectively separates rotenone from deguelin and from their respective Δ 6a(12a)-dehydro and 12a-hydroxy derivatives. Both computer-based predictions and preliminary experimental results indicate that this procedure will provide pure samples of all six of these compounds. The validity of the quantitative TLC procedure for determining rotenone and deguelin has been verified. Adoption of isopropanol as a solvent has reduced the time of the reduction step of the GLC procedure for the same determination from 2 hours to 15 minutes. Extracts of T. vogelii leaflets containing up to 60 percent rotenone plus deguelin were readily prepared on laboratory scale. Preliminary experiments suggest that efficient separation of leaflet from stem will be achievable by partial drying, differential mechanical reduction, and air or sieve classification.

C. Technology--Process and Product Development

1. Chemical pulps from kenaf. Preserving kenaf by freezing produced no significant changes in pulping characteristics or in the quality of derived sulfate pulp. Inclusion of foliage and stalk tops of green kenaf in silage preparation reduced yield of screened pulp and initial freeness of pulp although physical properties of pulps were similar. Storage by total immersion in water appeared to be more effective than ensiling (with or without additives like sulfuric acid or molasses), a soak-and-drain procedure or a wet-pile technique. Washing of products from all storage treatments except total immersion improved freeness of derived sulfate pulps. Washing following total immersion improved bleachability of the derived pulp with substantial retention of desirable strength characteristics. Washing of silages to remove silage acids and solubles from the fibrous material before pulping was more effective than pressing silage by laboratory techniques. On exploratory tests, removal of the plant juice by dewatering green kenaf with a screw press improved pH value of spent pulping liquor and bleachability of sulfate pulp.

RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS AND NATURALLY OCCURRING TOXINS

B. Microbiology and Fermentation

1. Tumor-inhibiting substances from plants. Extracts from seeds of 10 plant species were found to be "confirmed actives" in inhibition of one or

more tumor systems. These extracts are now being fractionated to concentrate the active principles therein. One of the extracts, which principally contains alkaloids, is active against lymphoid leukemia.

Publications - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Industrial Products

Chemical Composition, Physical Properties and Structure

- Bagby, M. O., and Mikolajczak, K. L. Dec. 5, 1967. Alkali isomerization of crepenynic acid to 8,10,12-octadecatrienoic acid. U.S. Patent 3,356,699.
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- Hagemann, J. M., Earle, F. R., Wolff, I. A., and Barclay, A. S.¹ (¹Crops Research Division, Beltsville, Maryland). 1967. Search for new industrial oils. XIV. Seed oils of Labiatae. Lipids 2(5), pp. 371-380.
- Kleiman, R., Davison, V. L., Earle, F. R., and Dutton, H. J. 1967. Determination of petroselinic acid by microreactor chromatography. Lipids 2(4), pp. 339-341.
- Kleiman, R., Miller, R. W., Earle, F. R., and Wolff, I. A. 1967. (S)-1,2-Diacyl-3-acetins: Optically active triglycerides from Euonymus verrucosus seed oil. Lipids 2(6), pp. 473-478.
- Kleiman, R., Spencer, G. F., Earle, F. R., and Wolff, I. A. 1967. Fatty acid composition of Ephedra campylopoda seed oil. Chem. Ind. (London) (21), pp. 1326-1327.
- Mikolajczak, K. L., Rogers, M. F., Smith, C. R., Jr., and Wolff, I. A. 1967. An octadecatrienoic acid from Lamium purpureum L. seed oil containing 5,6-allenic and trans-16-olefinic unsaturation. Biochem. J. 105(3), pp. 1245-1249.
- Mikolajczak, K. L., and Smith, C. R., Jr. 1967. Anomalous borohydride reduction of some α -acyloxy tosylhydrazones. Chem. Ind. (London) (51), pp. 2150-2151.
- Mikolajczak, K. L., and Smith, C. R., Jr. 1968. Penta-acid triglycerides of Chamaepeuce afra seed oil. Biochim. Biophys. Acta 152(2), pp. 244-254.

- Mikolajczak, K. L., Smith, C. R., Jr., and Wolff, I. A. 1968. Glyceride structure of Cardamine *impatiens* L. seed oil. *Lipids* 3(3), pp. 215-220.
- Miller, R. W., Earle, F. R., Wolff, I. A., and Barclay, A. S.¹ (¹Crops Research Division, Beltsville, Maryland). 1968. Search for new seed oils. XV. Oils of Boraginaceae. *Lipids* 3(1), pp. 43-45.
- Smith, C. R., Jr., Kleiman, R., and Wolff, I. A. 1968. *Caltha palustris* L. seed oil: A source of four fatty acids with cis-5-unsaturation. *Lipids* 3(1), pp. 37-42.
- Tallent, W. H., and Kleiman, R. 1968. Bis(trimethylsilyl)acetamide in the silylation of lipolysis products for gas-liquid chromatography. *J. Lipid Res.* 9(1), pp. 146-148.
- White, G. A.,¹ and Wolff, I. A. (¹Crops Research Division, Beltsville, Maryland). 1968. From wild plants to new crops in USA. *World Crops* 20(3), pp. 70-76.

Technology--Process and Product Development

- Clark, T. F., Uhr, S. C.,¹ and Wolff, I. A. (¹Hudson Pulp and Paper Corp., Palatka, Florida). 1967. A search for new fiber crops. X. Effect of plant maturity and location of growth on kenaf composition and pulping characteristics. *Tappi* 50(11), pp. 52A-56A.

FORAGE UTILIZATION (NORTHERN REGION)

USDA and Cooperative Programs

Location and Nature of Intramural Work	:Scientist Man-Years FY 1968:	
	: Research Problem Area	: Total
	407	
Peoria, Illinois		
<u>Feed</u>		
A. Microbiology and Toxicology	2.4	2.4
Total	2.4	2.4

Problems and Objectives

Tall fescue grass is grown extensively in the Southeast, in the Inter-mountain States, and in the Pacific Northwest as a forage crop for cattle and other domestic animals. It has excellent agronomic characteristics, producing well on marginal land and remaining green during cool weather when other grasses are dormant. Thirty-five to fifty million acres of fescue are grown for forage use in the Southeastern part of the United States alone. Cattle grazing on pasture that is predominately tall fescue sometimes develop a disease known as "fescue foot." In severe attacks, animals become emaciated and frequently die. Elimination of this disease would prevent an estimated average economic loss of about \$5 million annually.

The major objectives of current research are to discover the cause of fescue foot disease and to develop and evaluate alternate ways for its prevention.

Progress - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Feed

A. Microbiology and Toxicology

1. Fescue toxicity. The α -acetamido- Δ^8 -butenolide is toxic to cattle, but the production of fescue foot was not demonstrated. A fraction from Fusarium sp. NRRL 3249 (previously called F. nivale) grown on hay was toxic to the rabbit as was an extract of a culture grown on rice. After counter-current distribution fractionation of the extract from the rice culture, one or possibly two compounds showed toxicity to the mouse. No butenolide was detected in the fractions. Isovaleroloxo diacetoxyscirpenol, also a suspected toxin, was also undetected. A total of 171 aqueous-ethanolic extracts of cultures of molds isolated from a toxic pasture has been screened for toxicity in the mouse. Eighteen of 49 molds belonging to the genus Fusarium exhibited toxin production. One Alternaria culture also produced a toxic extract. Epicoccum nigrum produced toxins when cultured under six different conditions. At the request of the Northern Division, the University of California examined the Fusarium sp. NRRL 3249 and classified it as an atypical strain of F. tricinctum.

Publications - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Feed

Microbiology and Toxicology

Yates, S. G., Tookey, H. L., Ellis, J. J., and Burkhardt, H. J.¹ (¹Western Utilization Research and Development Division, Albany, California). 1968. Mycotoxins produced by Fusarium nivale isolated from tall fescue (Festuca arundinacea Schreb.). Phytochemistry 7(1), pp. 139-146.



